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TOWARD A MORE MODERN STATE

A

Survey of Agricultural Research
in Maryland

EIGHTY-THIRD ANNUAL REPORT

BULLETIN A-181

Volume II

OCTOBER 1973

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Vo. No. II of III

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AGRICULTURAL EXPERIMENT STATION

COLLEGE PARK

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COLLEGE PARK, MD.

*TO: The Governor of Maryland
The Board of Regents
and the President, University of Maryland*

The Maryland Agricultural Experiment Station research program is dedicated to the advancement of agricultural sciences for the benefit of the citizens of the State. The proper interpretation and application of this knowledge will enhance production of crops, poultry, livestock, and livestock products. Perhaps of equal importance, this knowledge will enable improved utilization, management and conservation of land, water, air and energy resources. Most importantly, much of this new knowledge will contribute to improved quality of life for people whether their residence is rural, suburban or urban.

The major program thrust is to improve efficiency of production of crops, animals and animal products. Such enhancement may be from varieties or species which give higher yields or better nutritive quality, which may be more resistant to diseases or insects, or which result in improvement rather than degradation of land and water resources, or from systems which require less labor or eliminate drudgery and hazards.

Production oriented research is followed by processing or utilization research, and this includes nutritional values for, and acceptability of, new products by people. Thus, agricultural research is not just for farmers or agribusinesses; it is the basis of fulfillment of food and fiber needs for all people. Agricultural sciences are also the basis of management of home gardens, landscaped yards, and recreational areas of parks, forests and golf courses.

Production agriculture has historically been the Station's primary concern. However, in recent years much effort has been focused on waste management, economic policies, marketing systems, nutritional and consumer needs, aquatic biology and pollution abatement.

In recent months supplies and prices of food, agriculture's role in world trade, the balance of payments and the energy requirements for producing and processing of crops have been front page news. Study of the summaries in this report will show a high level of relevancy between our research and current critical needs.

It is with pleasure that I transmit this Eighty-third Report of the Maryland Agricultural Experiment Station established at the University of Maryland in accord with requirements of Acts of Congress, March 2, 1887. Reported here is research for the period July 1, 1969 - June 30, 1973, and a statement of receipts and disbursements for the same period.

Respectfully submitted,

R. L. Green
Acting Director

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*Contribution No. 4871 of the Maryland Agricultural Experiment Station
Ms. Rec'd. 5-73.*



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PROJECT REPORTS

**AGRICULTURAL
and
EXTENSION EDUCATION**

RELATIONSHIP OF TEACHER ATTITUDES TO STUDENT OCCUPATIONAL SUCCESS

The objectives of the study were: 1) To determine what effect teacher attitudes toward the world of work, vocational education, teaching and students have on the occupational success of their former students; 2) To determine what effect teacher attitudes toward the world of work, vocational education, teaching and students have on the occupational satisfaction of their former students.

Positive and negative attitude teachers included in the study were selected on the basis of their expressed attitudes as perceived by teacher educators and/or supervisors of agricultural education. The sample consisted of 53 teachers in a four-state area which included Maryland, Virginia, Pennsylvania and Delaware. The total number of teachers included in the analysis was 35 - 20 teachers were considered to have positive attitudes while 15 teachers were considered to have negative attitudes toward the world of work, vocational education, teaching and students.

The former student sample consisted of 71 former students of the positive and negative attitude teachers who had at least three years of vocational agriculture with the teacher, had not been out of school more than one year, and were working in a non-farm occupation. A total of 28 former students were included in the analysis, 19 from the positive group of teachers, and nine from the negative group of teachers.

The supervisor sample contained 28 job supervisors of the former students. A total of 22 supervisor ratings were included in the analysis, 15 ratings on former students of positive attitude teachers and seven ratings on former students of negative attitude teachers.

Occupational success was measured through the use of supervisor ratings of the former students' job performance.

Occupational satisfaction was measured through the use of the Job Descriptive Index, which was completed by the former students.

Data analysis was through the use of descriptive statistics, t-test and the Mann-Whitney U Test.

FINDINGS

1. Negative attitude teachers were older.
2. Negative attitude teachers had been teaching vocational agriculture more years.
3. There was no difference in the number of years teachers have been teaching in their present position.
4. More teachers in the positive group had taught vo-ag in another school prior to their present position.
5. Teachers in the negative group in general were in schools with lower enrollment.
6. More teachers in the positive group offered students a greater diversified vo-ag program.
7. Former students of positive attitude teachers had a higher mean success rating, however, it was not significant.
8. Former students of negative attitude teachers had a higher occupational satisfaction score; however, it was not significant.

9. Former students of positive attitude teachers had a slightly higher mean age.
10. More former students of negative attitude teachers had held a full-time job while attending high school.
11. The average number of jobs held since graduating from high school was greater for the former students of positive attitude teachers.
12. All students included in the analysis could be categorized into one of nine job categories: machine trades, mechanic, laborer, nursery or greenhouse worker, clerk, engineering aide, truck driver, construction and service station attendant.
13. Former students of positive attitude teachers were employed in more of the nine job categories.
14. The job category containing the most former students in both groups was laborer.

CONCLUSIONS

The data did not support the following hypotheses:

1. Students experience greater subsequent occupational success when taught by teachers with more positive attitudes toward the world of work, vocational education, teaching and students.
2. Students experience greater subsequent occupational satisfaction when taught by teachers with more positive attitudes toward the world of work, vocational education, teaching and students.

Project No. T-20

COMMUNITY SERVICES FOR NONMETROPOLITAN PEOPLE IN THE NORTHEAST

Nine of the 13 Northeastern states are cooperating in this regional research project. The first 18 months of the project were utilized in project design, secondary data analyses and planning for primary data collection. The year of 1973 will involve primary data collection on human services delivery in the areas of social services, health and education.

The project design calls for researching services delivery within a social systems theoretical model. Communities, families, counties, delivery organizations and state and federal agency components are looked upon as subunits in the total social system involved in delivery of services to families in rural communities.

Primary data will be collected on delivery organizations and from families on the factors that determine the type, quantity, quality and distribution equity of services delivered to nonmetropolitan families. The major variables will focus upon the systemic nature and mix of services being delivered in the various study communities throughout the Northeast. The uniqueness of the project will be in the focus on the mix of services in a systemic framework rather than the usual approach of studying individual services.

It is anticipated that each state will report on the community area(s) studied in their state. There will also be one or more regional publications which compare the differences attributable to the different social systems found throughout the Northeast region.

Project No. Md. T-21 (NC-77)

PATHS OUT OF POVERTY

This is a Northeast regional research project directed (1) at the relationships between families in poverty and programs designed to help them climb out of impoverishment, and (2) the design of improvements in programs to more effectively meet the needs of the impoverished.

The Maryland contributing project involved the collaboration of Delaware, Maine and Pennsylvania for data from the three states in addition to Maryland and thereby have more generalizable results as well as number of cases. The project focused on the use of paraprofessional aides in the Nutrition Education programs conducted by Cooperative Extension Services in those states.

The research design views the aides as a linkage position between the families in poverty, Extension Service knowledge on nutrition and access to other services such as health, manpower training, employment, housing, family planning and legal services through referrals to other agencies.

To assure that the research adds to basic social science knowledge, as well as to applied research interests, a six function theory of leadership was utilized for the project design and data instruments for researching the linkage chain from families through the aides to Extension and other agency services.

Data on 72 Maryland aides and their 16 supervisors were gathered in the spring of 1971. During the summer and fall of 1971, data was collected on 100 aides and 29 supervisors in Pennsylvania and 77 aides and 17 supervisors in Maine. These data were followed in the spring of 1972 with data on 24 aides and 2 supervisors in Delaware. Cases for aides is 272 and for supervisors 64. The summer and fall of 1972 and the spring of 1973 have been utilized in data processing, computer program writing and testing and preparations for data analysis and report writing.

The first publication from the project was a paper at a professional meeting, followed by Maryland Agricultural Experiment Station Miscellaneous Publication 821, *Professional and Paraprofessional Role Differentiation* in January, 1973. Future publications are to include a master's thesis, a methodology report, a descriptive results report, at least one professional journal article and a final analytical scientific bulletin.

Project No. Md. T-18 (NE-68)

PROJECT REPORTS

AGRONOMY

This report gives the research in progress in the Department of Agronomy. The research program in the Department includes the development of improved crop varieties and improved soil and crop management practices that result in more efficient production. Considerable emphasis is also given to research on soil and water conservation, soil and water use, soil-water pollution, air pollution and the disposal of waste materials.

The wide range of soils and 1.9 million acres of agronomic crops (corn, soybeans, tobacco, small grain, turfgrasses, hay and pasture) grown in the state, with a value of approximately 175 million dollars, emphasize the size and importance of the research program in agronomy. To solve crops and soils problems, research work is conducted at University Laboratories, on outlying Experiment Station Farms, cooperatively with farmers, golf course superintendents and others throughout the state. The help and cooperation received from the many people in the state in carrying out our research program is greatly appreciated.

SOIL TESTING FOR ENVIRONMENTAL CONTROL

Copper is an element that may cause toxicity to plants if the amount that accumulates in soils is great enough. Most soils have received copper from "Bordeaux mixture" sprays, minor elements in fertilizer, manure, etc. The copper in sewage concentrates in the sludge so that additions of sewage sludge to soil results in a vigorous copper application. Since copper moves only short distances in soils, every one of these treatments adds to the total amount of copper present in the soil. If copper in Sassafras sandy loam is over 225 lbs/acre, yields will decrease (if organic matter level is normal). If it is 300 to 375 lbs/acre in the mattapex, yields will decrease. When these copper levels are further increased, yields will drop, but more rapidly.

Project No. O-82

DEVELOPING, IMPROVING, AND CALIBRATING SOIL TESTS

The chemistry and toxicity of arsenic in Maryland soils, as well as many soils throughout the U.S., were studied. Arsenic was found to accumulate as iron, aluminum and calcium compounds in soils. The phytotoxicity of arsenic in soils depends on its chemical form. Sodium arsenate, a water soluble form, is most toxic; iron arsenate, a relatively insoluble form, is least toxic; and calcium and aluminum arsenates are intermediate in their toxicities.

Soil test extraction solutions used to determine the presence of toxic arsenic were proven and calibrated. Four of the tests studied were equally good. They included the extracting solution now in use in the Maryland State soil test laboratory ($r = .81$). To arrive at many of these results, new tests were developed which were made available to scientists throughout the world. They include calcium determinations using a specific ion electrode, and determination of arsenic toxicity by summing the amount of iron, aluminum, calcium and water soluble arsenic in the soil as modified by their individual toxicity ($r = .82$). Another test developed under this project was clay identification by a density gradient method.

This project was terminated June 30, 1972.

Project No. O-78

MORE EFFECTIVE USE OF SOIL AND FERTILIZER NITROGEN

The study of the fate of nitrogen in soils has been improved by the use of the expensive ^{15}N isotope of nitrogen. Its use on a normal size corn experiment would cost \$300,000+. As this amount of money is unthinkable, a micro (very small) ^{15}N type of experimentation was developed to be used in conjunction with a normal size nitrogen fertilizer field study. This resulted in a more than 300+ fold reduction in material costs.

Results of studies from such an experiment showed that when 240 lbs. of nitrogen was added to a heavy silt loam, 28 percent of the added nitrogen was left in the soil at harvest time, 19 percent was in the harvested plant and 52 percent of the added nitrogen was lost. This can be compared to a 30 lbs. nitrogen/acre treatment. Here, 13 percent remained in the soil, 49 percent was in the plant and 48 percent of the added nitrogen was lost.

Additional studies on these heavy silt soils showed nitrogen did not go too deep into the soil before it was apparently lost to the atmosphere. Usually less than three feet of silty soil on Maryland's Eastern Shore was required to affect this loss. Results of this type show that fertilizer nitrogen, applied to corn on silt loam soils of the Eastern Shore, does not appreciably enter into the ground water.

A study of plant uptake and release of ammonia, from and to the atmosphere, showed conclusively for the first time that ammonia in the average atmosphere is used by plants. Aerial portions of corn, pigweed, pine and soybean plants absorbed $^{15}\text{N-NH}_3$ at a concentration of $22\text{Ug NH}_3/\text{M}^3$. Soybeans and corn plants were more effective than pigweed in this type of absorption. These results show conclusively that plants are definitely an atmospheric sink for NH_3 in average air.

Project No. O-57

MICRO-NUTRIENT REQUIREMENTS OF CORN ON IMPORTANT MARYLAND SOILS

Applications of zinc manganese and boron were made to soils on which the previous corn crops had exhibited zinc deficiency symptoms. Only the results of two of seven field experiments showed yield increases from micro element treatment. The corn yields on an othello soil (pH 7.1) with 0.8 lbs/acre of available zinc were increased from 86 to 110 bu/acre by five lbs/acre of zinc. Additional amounts of zinc fertilizer gave no further yield increase. Protein content of this grain was increased significantly from 10.25 to 10.81 by zinc treatments. When plant tissue tests were compared with corn yields, a higher correlation was obtained with the ratio of the equivalents of H_2PO_4^- to Zinc^{++} in the corn ear leaf than with zinc content alone.

Soybean yields were not increased on this soil by zinc treatments which had increased corn yields, but soybean yields were increased by 12 lbs/acre of manganese.

A Beltsville loam which contained 2.6 lbs/acre of zinc and 86 lbs/acre manganese responded to a combination of zinc plus boron fertilizer. The yield of the untreated area was 53 bu/acre while that of the treated was 81 bu/acre with an accompanying increase in grain protein from 8.9 to 11.7 percent.

This project was terminated June 30, 1970.

Project No. O-62

TOBACCO BREEDING, TESTING AND QUALITY EVALUATION OF MARYLAND TOBACCO

Maryland ten (Md. 10), a mosaic resistant variety of Maryland tobacco, was released jointly by the U. S. Department of Agriculture and the Maryland Agricultural Experiment Station in 1969. The new variety was derived from a cross of RMW (a mosaic resistant burley-type breeding line) and Catterton (a standard and quite popular variety of Maryland tobacco). Six backcrosses to Catterton and ten generations of selfing followed the original cross prior to release. Plant, chemical and physical traits of Md. 10 are similar to Catterton, the recurrent parent. Md. 10 is average in yield of cured leaf per acre, has a good quality leaf and is a rather tall medium broadleaf type. Md. 10 is generally about two days later flowering than Catterton. Total alkaloids, total nitrogen, burning ability and filling capacity are the same as Catterton.

Since 1965, the popularity of tobacco varieties developed by the Maryland Agricultural Experiment Station and the U. S. Department of Agriculture has increased greatly. In 1966, 18 percent of the seed distributed by the Maryland Tobacco Improvement Foundation was comprised of Md. 59 and Md. 609, the only two released varieties at that time. In 1971, however, 72 percent of the seed distribution was comprised of the released varieties Md. 59, Md. 609, Md. 64 and Md.

10. The most popular variety in 1971 was Md. 609. In fact, 53.3 percent of the total 13,653 ounces of seed distributed by the Maryland Tobacco Improvement Foundation was of this variety. The rapid acceptance of new varieties indicates the importance of the tobacco breeding program to farmers in Maryland.

Each year replicated variety trials were conducted at three locations to study the response of Maryland tobacco varieties and advanced breeding lines to different soil and climatic conditions. Breeding lines with disease resistance were evaluated and compared with the standard varieties. In general, Md. 64 was the highest yielding variety. While Md. 609 exhibited the best quality. However, in recent tests, several breeding lines have yielded more cured leaf per acre than Md. 64 while equalling Md. 609 in quality.

Location differences for the various varieties and breeding lines in the variety trials are observed each year. However, trends are difficult to establish because of the variation in total rainfall and particularly its distribution during the summer months.

Four breeding lines (J37, J201, J231, and M872) which had performed very well in the variety trials for two years were evaluated visually and chemically by foreign and domestic tobacco companies in 1972. Based on variety trial data and company evaluations, two of these breeding lines appear to have real potential as new varieties and may be released in the near future.

Md. 609, the most popular variety at the present time, appears to lodge more than the other standard varieties. Results from a two year greenhouse study showed that Md. 609 had the smallest root system of all the varieties and Wilson had the largest. Several breeding lines were included in the study, and some lines with Md. 609 in their pedigree exhibited larger root systems than Md. 609. Therefore, it appears that lodging resistance can be increased by breeding for larger root systems.

A two year hybrid study involving a five-parent and a seven-parent diallel set of crosses was completed in 1971. A small but significant heterotic response was found in four out of ten traits studied. The hybrids flowered earlier, had a higher yield and dollar return per acre and per hundredweight than the parents (varieties). The range in heterosis was from -4 percent for days to flower to 8.5 percent for dollars per acre. When heterosis was observed in the F_1 generation, inbreeding depression also was observed in the F_2 generation. In comparing the relative magnitude of general combining ability versus specific combining ability, a large part of the total genetic variance was associated with general combining ability.

Two studies have recently been completed in order to determine the effects and use of male-sterility *per se* and in combination with hybridization in Maryland tobacco. The first study involved the *Nicotiana megalosiphon* source of cytoplasmic male-sterility only. The objectives were to determine the effects of the *N. megalosiphon* cytoplasm on certain agronomic, chemical and growth traits of Maryland tobacco, and to determine the potential use of this source of male-sterility in producing hybrid seed. In this study five male-sterile varieties, ten male-sterile hybrids and their normal fertile counterparts were used. No significant heterotic response was found for the various traits studied. However, the male-steriles were lower in yield, value and plant height, had fewer leaves per plant, shorter internodes, a higher total nitrogen content and less weather fleck damage, flowered later and grew slower than the fertiles. The depressing effects of male-sterility were also observed with hybridization. The male-sterile hybrids were lower yielding, shorter in height, had fewer leaves, a higher total nitrogen content, less weather fleck damage and flowered later than the fertile varieties.

The second study involved the cytoplasmic male-sterile factor obtained from five *Nicotiana* species: *N. plumbaginifolia*, *N. undulata*, *N. megalosiphon*, *N. suaveolens* and *N. bigelovii*. Within each cytoplasm three male-sterile varieties, three male-sterile hybrids and their male-fertile counter-

parts were used. Results from this study indicated that the *plumbaginifolia* and *bigelovii* male-sterile cytoplasms caused a greater reduction in yield and value per acre than did the other three cytoplasms. The slight heterotic responses observed were generally eliminated by the male-sterile cytoplasms.

From these studies, it appears that the depressing effects of male-sterility offset any advantages of hybrids over the standard varieties. Therefore, breeding procedures appropriate for pure line development appear to be the most practical approach in Maryland tobacco.

Weather fleck, a tobacco leaf spot caused by air pollution, causes some damage in the Maryland tobacco producing area each year. A study was initiated in 1970 to determine the extent of genetic variability among several cultivars of Maryland and cigar types of tobacco, and to obtain estimates of general and specific combining ability. Seven varieties and 21 F_1 hybrids were grown at three locations for two years, 1970 and 1971. During August, data were collected on leaf damage caused by air pollutants, primarily ozone. Comparison of variety and F_1 hybrid performance indicated no significant amounts of heterotic resistance to weather fleck. Bel-B and Md. 64 were the most resistant varieties and Bel-W3 and Md. 59 were the most susceptible. Diallel analyses of variance of the F_1 hybrid generation suggested an appreciable amount of variance due to general combining ability, and the absence of variance due to specific combining ability. Genetic effects were stable with respect to locations and years. The genotype x environment interactions for the six environments were small. The predominance of additive genetic variance in this material suggests that progress could be made through selection. Phenotypic correlations of weather fleck with several agronomic traits suggest that varieties which are more susceptible to weather fleck generally flower early, are tall in plant stature and have wide leaf spacings on the stalk.

A two year study on varietal response to mechanical harvesting was conducted in 1969 and 1970. Seven varieties and two breeding lines were used to evaluate mechanical harvesting with the conventional hand method. Plants harvested by machine were lower in quality, taller, had more leaves and greater leaf loss than when harvested by hand. No differences were observed between the two harvest methods for yield and value per acre. The lack of significance of the variety x harvest method interaction suggests that all varieties and breeding lines performed relatively the same for both harvest methods. Yield and value per acre increased with larger leaves and later flowering. However, when leaf size, leaf angle and flowering increased, there was a trend towards greater leaf loss.

For many years, tobacco farmers in Maryland saved their own seed from year to year. Therefore, a considerable number of farmer selections arose over a period of years. A study was completed recently in order to determine the amount of variability present in six Catterton-type farmer selections. In general, the agronomic and chemical performance of the farmer selections were very similar when compared among the selections or with Catterton. Significant differences among the various selections were observed for only two traits – plant height and internode length. Since no useable variation existed among the farmer selections tested, it appears that genetic variation must be obtained by some other means such as hybridization with other varieties.

Project No. B-103

LEACHING OF NITRATE NITROGEN FROM SEVERAL FERTILIZER SOURCES WHEN APPLIED TO SOILS OF VARIOUS TEXTURES

A comparison was made of the effects of four nitrogen sources on soil nitrate levels, location of nitrate concentrations in the soil profile during the season, response of corn and small grains, and nitrogen residues carried over from one season to the next. The nitrogen sources included ammonium nitrate, urea, nitrate of soda and sulfur-coated-urea.

Small grain yields indicated that sulfur-coated-urea, a slow release form of N, was inferior on small grains since it apparently did not release N fast enough or at the proper time. The highest grain yields were obtained when a readily available N source was topdressed in late February or early March just prior to the period of greatest demand. Nitrogen applied in the fall or winter, regardless of source or method of application, produced significantly lower yields than early spring applied N from a readily soluble source.

Corn grain and silage yields from plots receiving up to 480 lbs/A of nitrogen plowed down in the spring indicated that, in general, the various N sources were comparable except that silage yields were lower from the nitrate of soda. In 1972, residual N in plots which had received 480 lbs. N/A from slowly available sulfur-coated-urea in 1971 was sufficient to nearly double the grain yields compared to other sources applied at the same rate in 1971. This demonstrated that sulfur-coated-urea did not break down as fast as the soluble sources and held some promise of reducing soil nitrogen losses from leaching and volatilization.

Soil nitrate N concentrations were extremely variable throughout the year. In general, nitrate levels from sulfur-coated-urea were much lower during the first several months following application than were nitrate levels from the other nitrogen sources. By late fall, nitrate levels could often still be detected under sulfur-coated-urea plots, but ranged from very high to very low for the other sources, depending upon weather and soil drainage conditions.

Although nitrogen from sulfur-coated-urea did not move out of the soil as rapidly as nitrogen from more readily available sources, there was no apparent economic advantage to this slow release form on small grain or corn. It is hoped that further research will produce a satisfactory material which can be applied to small grains in the fall, eliminating traditional spring topdressing, or in the spring to corn on light sandy soil, eliminating necessary nitrogen sidedressing.

Project No. O-80

THE INFLUENCE OF SOIL ORGANIC NITROGEN ON THE GROWTH OF SOYBEANS

Fertilizer nitrogen addition to soybeans generally decreases the amount of nitrogen fixed by the plant. Yield response which is variable will depend on nodulation, soil nitrogen level and season. The effectiveness of "organic nitrogen" was studied as a means of increasing yield by comparing fertilizer nitrogen applied to a previous rye crop with nitrogen applied at soybean planting in 1970 and 1971. Nitrogen was applied at 0, 50, 100 and 200 lbs. N/acre either on a previous rye crop or at soybean planting. Two carriers, ammonium nitrate or urea, were used. Significant yield differences were not observed in 1970. However, nitrogen applied to the rye in 1971 significantly increased soybean yield two bu/acre when compared to nitrogen applied at soybean planting. Soybean yield responses from rye-applied nitrogen were obtained from ammonium nitrate and not urea. Soybean nitrogen fixation rate was stimulated throughout the season by the addition of 50 lbs. nitrogen applied to the previous rye crop. Stimulation of nitrogen fixation rate late in the season was obtained from rye-applied nitrogen at rates to 200 lbs/acre. These results suggest the nitrogen applied in an organic form may stimulate the soybean plant to fix more nitrogen which, in turn, may produce yield increases. Such observations were not obtained from inorganic nitrogen applied directly to soybeans at planting.

Project No. O-11

CONTROL OF JOHNSONGRASS FROM SEED AND RHIZOMES

Laboratory assay has indicated that the thiocarbamate herbicides plus the protectant N. N-diallyl-2,2-dichloroacetamide may be effective in controlling Johnsongrass in corn. A randomized complete block field experiment was initiated on an established Johnsongrass stand planted to corn. Thiocarbamate herbicides used in this study were found to have a significant effect on Johnsongrass control as measured by visual ratings. The best treatment was EPTC at three times the normal rate (9 lb/A). This gave 86 percent Johnsongrass control at the end of the season. At two times the normal rate EPTC control of Johnsongrass was 80 percent on 6/2/72. This control was equal to the control caused by equivalent rates of vernolate and 10 percent greater (not significant) than butylate. Butylate at normal rates (4 lb/A) was consistently the poorest treatment. Both corn yield and weight of Johnsongrass indicated that three times the normal rate of EPTC gave the best control of all thiocarbamate treatments. From one year's data it appears that the thiocarbamate herbicides have an excellent potential for Johnsongrass control in corn.

Project No. B-95

FOREST BUFFER STRIPS IN CONTROLLING ANIMAL WASTE RUNOFF INTO STREAMS

Monitoring for stream quality on the University of Maryland Forage Research Farm in Howard County has been conducted since July 1, 1972. Approximately 25 samples are taken at two week intervals from tributaries on the research farm that lead into the Middle Patuxent River. Samples are routinely monitored for nitrate nitrogen, total phosphorus, total and fecal coliform and fecal streptococci.

One forest and one grassland site are under study in regard to animal waste runoff into streams. Special samplers have been constructed to record surface runoff and to remove a sample for analysis. These samplers have been designed to operate continuously on spring-wound motors which eliminate the need for electrical current.

The forested area for study is a part of a watershed that contains a small stream. Analysis of this stream for fecal coliform has indicated that it is relatively free from contamination. In the forested area, animal waste will be applied on a grass area above a forest watershed. Surface and subsurface movement of fecal coliform, nitrate and phosphate will be monitored below the application area. Streams will be monitored through the watershed for any evidence of contamination.

The grass location has been selected in a relatively uniform area where a length of 200 feet of 12 percent slope is available for study. Duplicate waste treatments, plus controls, will enable statistical evaluation of the plot area. Waste samplers placed at approximately 25, 50, 100 and 200 feet downward from the waste application area will provide runoff water for analysis. The effectiveness of the buffer strips in minimizing waste movement will be evaluated.

Project No. BO-1

PRINCIPLES OF DISSIPATION AND MOVEMENT OF TRIAZINE AND OTHER HERBICIDES

Canada thistle (*Cirsium arvense* L.) plants, containing one elongated and one recently emerged shoot from the same root segment, were treated with ^{14}C -ring labeled 2-chloro-4-(ethylamino)-6-(isopropylamino)-2-triazine. Fourteen days after atrazine application to the elongated shoot, 95 percent of the recovered activity remained in this shoot. The distribution pattern of ^{14}C suggested

movement with the transpiration stream. 80 percent of ^{14}C in the treated shoot was in the form of unaltered atrazine 14 days after application. Greenhouse studies with non-labeled atrazine indicated that this herbicide had only an indirect effect on portions of the plant located basipetally to the area of application.

2-chloro-4-(ethylamino)-6-isopropylamino-s-triazine (atrazine) volatility from glass, plant and soil surfaces was determined by passing the air above the experimental material through florasil columns. Percent of atrazine volatility was estimated by counting the ^{14}C -atrazine tracer present in the column eluate and that which remained on the treated surface. Volatility from 125 ml glass erylenmeyer flasks was influenced by temperature, air velocity, purity of the atrazine and, most important, the amount of atrazine present. The highest percentage of volatility was recorded at 40 C with an air velocity of 2000 ml/min. Under these conditions, 90 percent of the one ug of atrazine applied to the flask was located in the florasil column at the end of 48 hours. The lowest volatility recorded from glass surfaces was when 1000 ug of atrazine was added to the flask. At the end of 48 hours, less than 20 percent of the atrazine applied was located in the florasil column. From 18-27 percent of the atrazine applied to dried plant tissue was lost through volatility at 40 C in 48 hours. Atrazine was applied to this tissue by soaking the tissue in a solution of atrazine at a concentration similar to that used in the field (37 mM). Droplets of atrazine (1 ul), at a concentration of 37 mM, were applied to the surface of intact leaves. Two days after treatment 50 percent of the activity was located in the florasil column. Volatility of atrazine from the soil was less than ten percent in 48 hours at 40 C under all conditions tested. It was concluded that atrazine volatility is a major factor of atrazine dissipation when applied to foliage, but not when applied to the soil.

Project No. B-121

COMPOUNDS IN WEEDS RESPONSIBLE FOR DORMANCY AND THE REDUCTION OF CROP YIELDS

A germination and growth inhibitor was exuded from Johnsongrass rhizome segments incubated in distilled water at five, 25, or 30 C for seven days. However, inhibitory properties were most pronounced at five C. The inhibitor was bioassayed using the following plant species: wheat, barley, soybeans, tomato and Japanese millet. The inhibitor in the rhizome exudate reduced the percentage germination of cotton, delayed germination of barley and cotton, inhibited root growth of barley, cotton, Japanese millet and tomato, and inhibited shoot growth of barley, corn, Japanese millet and tomato.

The presence of shurrin in rhizome exudate was confirmed by analysis of its degradation products, p-hydroxybenzaldehyde, HCN and glucose, following either 1 N HCl, 0.1 N NaOH, or glucosidase (emulsin) hydrolysis of chromatographically purified extracts. Following emulsin hydrolysis for 4 hours, glucose, p-hydroxybenzaldehyde and HCN were formed in a 1:1:1 molar ratio which is characteristic of the enzymatic degradation of dhurrin. Only 8.5 percent of the total quantity of dhurrin present in Johnsongrass rhizomes was collected in the exudate following incubation of five C for seven days.

This project was terminated June 30, 1972.

Project No. B-115

EFFECTS OF PRODUCTION METHODS ON CORN SILAGE

Corn silage yields continue to be pushed upward through the use of better varieties and higher fertilizer applications. It is important to investigate the possible effects of high levels of fertilizer on the indigestible components of the silage. In this study, the fiber, lignin and mineral contents of

corn grown under high levels of various fertilizer nutrients were studied. This work has shown that the mineral content of the silage can be altered substantially by adjusting the soil fertilizer levels. It has also been shown that high levels of nitrogen and potassium fertilizer result in high digestible dry matter percentages.

In addition to changes in digestibility of the silage, nitrogen plus magnesium as the only added fertilizer materials resulted in significantly higher plant lodging. These findings assume greater importance as yield plateaus are reached and the quality of corn silage becomes of greater importance.

This project was terminated June 30, 1972.

Project No. B-114

RESPONSE OF CROPS TO FERTILITY AND IRRIGATION

Despite the fact that Maryland is located in an area commonly referred to as the "Humid East," drought is our most frequent and unpredictable crop hazard. Irrigation can effectively solve this problem, but the cost is high and yield increases obtained by irrigation usually do not make it economical for field crops. Changes in cropping practices, however, might make irrigation more favorable from an economical standpoint. One way to increase the intensity of crop production is by double cropping, but with this practice the first crop usually depletes the soil moisture to such an extent that irrigation is vital to the second crop. In this study, comparisons were made with both single and double cropping practices, and under both irrigated and non-irrigated conditions. Fertilizer levels were also studied to determine optimum levels for different cropping patterns with and without irrigation. The cropping patterns studied were corn grain-winter fallow, corn silage-winter fallow, corn silage-barley silage and soybeans-barley grain.

In the 1970 growing season there was abundant rainfall from April through July, and irrigation water was not needed until August. As a result, there was only a slight crop response to irrigation. Double cropping outyielded single cropping only at the high fertility level and then only slightly. Corn under high fertility yielded 30 bushels more than under low fertility. Silage crop yield differences between high and low fertility were less than with grain.

Rainfall was low in April, 1971, and 1.25 inches of supplemental irrigation was applied. An additional three inches of irrigation was added in early July to supplement the scant rainfall of late June and early July. Heavy

rainfall was recorded during the remainder of the growing season, and only 1.5 inches of irrigation water was applied in August. Because of the abundant rainfall, there was no irrigation response in grain yield and a slight yield increase with the silage crop. As in the previous year, high fertility increased yields over low fertility.

In the previous two years of this experiment, yields from double cropping under high fertility and irrigation substantially outstripped non-irrigated treatments. In the most recent two years, with high natural rainfall, substantial differences were not observed.

This project was terminated June 30, 1972.

Project No. B-113



In a dry season, irrigation can boost yields of a summer crop which follows a winter grain.

CONTROLLED FLAMING IMPROVES MIDLAND BERMUDAGRASS YIELDS AND FORAGE QUALITY

Effects of controlled stubble flaming, sod seeding, nitrogen rates and nitrogen source on winter annual weed control, forage yields and forage quality of Midland bermudagrass swards were studied for two years.

There were fewer winter annual weeds on those plots flamed in 1970 and on those plots with the sod-seeded cereals. The reason for this reduction appeared to be associated with a reduction in weed seed production. The direct effects of controlled flaming and sod-seeding were similar to those obtained in 1970. Sod seeding again produced the largest amount of weed-free forage. Flaming was effective in controlling weeds, and it appeared that once a schedule could be established, flaming each year would not be necessary for satisfactory weed control. Flaming did improve the efficiency of urea nitrogen fertilization slightly. This was not true for ammonium nitrate.

'Penngift' and 'Chemung' crownvetch varieties grown with 'Ky-31' tall fescue and harvested differentially for two years were uniformly harvested twice in 1972 to measure residual treatment effect. Penngift crownvetch was more persistent than Chemung under all harvest managements and, as a result, forage yields were significantly higher for the Penngift mixtures. In terms of persistence, early bloom (four cuts) was least desirable. Delaying the first harvest until late bloom resulted in greatest stand persistence. Frequent harvest did not appear to be detrimental, providing a good supply of active axillary buds were retained. It appears, therefore, that crownvetch-grass mixtures could be successfully maintained under continuous grazing, providing the intensity of grazing is not severe enough to remove these active buds. Crownvetch appeared to be better adapted to a pasture than to a hay-type management.

Project No. B-119

BETTER PASTURES FOR BEEF CATTLE

There are a number of pasture combinations that can be used in beef cattle operations in Maryland. The most common and widely used pasture is Kentucky bluegrass white clover. When properly fertilized and graxed, this pasture will produce 90 to 95 percent as much animal products as taller growing species, such as orchardgrass-ladino clover. However, bluegrass cannot be used for hay or silage. It can only be pastured. This means that no operation should have more bluegrass pasture acreage than can be completely utilized during the flush spring growth when more than 75 percent of total production occurs.

In contrast, Midland bermudagrass makes most of its growth during the year when bluegrass is least productive. Where these two species have been alternately grazed in a pasture system, higher total production per acre has resulted, but more importantly, feed supply through pasturage has been extended over a greater portion of the year. Thus, the cost of maintaining a beef cow and her calf can be reduced. This and numerous other pasture combinations have been tested under this research project.

This project was terminated June 30, 1970.

Project No. B-56-J

MIDLAND BERMUDAGRASS CAN PRODUCE HIGH YIELDS OF QUALITY HAY

There is no perennial forage grass adapted to the central or southern Maryland counties that will produce more summer pasture, or more beef per acre, than Midland bermudagrass. When this species is utilized along with Kentucky bluegrass pastures, or when cereals are seeded into the Midland for winter pasture, the overall production is improved.

Recent research has demonstrated that Midland bermudagrass can be utilized as hay or silage. Hay yields in excess of ten tons per acre are easily obtainable with nitrogen fertilization of 300 to 400 lbs/A. The quality of this forage as measured by chemical analysis, animal digestion and intake studies, increased as nitrogen levels increased to around 400 lbs. N/A and as the forage was harvested at earlier stages of growth. Yields were lowered with more frequent harvesting. For hay management, it appears that Midland should be cut when it reaches a height of 15 to 18 inches. This will require harvest schedules between three and four weeks. Harvest should never be delayed until bottom leaves start to yellow and die if quality is to be maintained.

This project was terminated June 30, 1970.

Project No. B-74

FORAGE PRODUCTION IMPROVED WITH SOD-SEEDING

With time, many perennial forage stands decrease in both yield and forage quality. Many of these grasslands are located on land too steep for normal tillage operations, so management to maintain production and quality of these fields is all important. The sod-seeding technique (seeding improved species directly into the old sod without complete destruction of that sod) is an excellent way to improve or maintain yield and quality.

The sod-seeding technique has also been used experimentally to extend the production period of some species. For instance, the productive period of a Midland bermudagrass pasture can be extended two or more months into the winter and at least ten weeks earlier in the spring. This can significantly reduce the cost of livestock production. Annuals, seeded into a Midland bermudagrass sward, have been more productive and more easily managed than perennials seeded into those same sods.

This project was terminated June 30, 1970.

Project No. B-75

FIELD SOIL TEMPERATURE STUDIES

Cool-season turf species were maintained in a healthy, vigorous condition where soil temperatures were maintained lower than ambient conditions during the hot summer months. Greater root reserves and tiller development were major reasons for the improved turf quality. It was, likewise, possible to maintain a desirable green turf of tufcote bermudagrass (a warm-season species) into the winter months with air temperatures far below freezing for extended periods.

In 1971, a short-season corn (Funk G-17A) was grown at 77,511 plants/ha on field plots with soil temperatures of 10 deg. C, 21 deg. C, 32 deg. C, and ambient. Three fertility levels (112-0-0, 112-112-224, and 112-224-448 kg/ha of N-P₂O₅-K₂O) were worked into the top 10-cm of soil just prior to transplanting uniform corn seedlings (3-leaf stage) into field plots. Soil temperature differentials were established prior to transplanting. A 5-cm layer of straw mulch was placed on all

treatments to help maintain soil temperature differentials. Temperature plots were differentially irrigated to maintain "uniform" soil moisture on all treatments. Weekly measurements of plant height and leaf development were recorded. Records were kept on maturity dates, and all plots were harvested at mid-dent except the 10 deg. C plots which were harvested along with the last treatment of the 21 deg. C soil temperature plots.

Plant growth, as measured in terms of plant height, leaf development and maturation, was markedly affected by soil temperature and, to a lesser degree, by fertility level. Most rapid development occurred at 32 deg. C soil temperature at the high fertility rate. No soil fertility response was obtained at 10 deg. C. Plant fertility response did not occur until the soil temperature of the 10 deg. C plots was increased to 15 deg. C. Calcium deficiency symptoms were observed on the high soil temperature plots. This tended to be more severe at the high P-K fertility levels.

The following season, early, medium and late corn varieties were grown at the high fertility level used in 1971. Plant population and general plot treatment was similar to 1971.

The performance of 17A was similar to that recorded in the 1971 study at the high P-K fertility level. The varieties responded differently to soil temperature. The increase in development caused by soil temperature was greater with the later maturing varieties. Differences between varieties were greatest at 32 deg. C and least at 16 deg. C. Ear height at harvest was highest at 24 deg. C or ambient and lowest at 32 deg. C, with 15 deg. C being intermediate. Ear height generally increased with later maturing varieties. Below ambient temperatures reduced grain and total dry matter yields more than temperatures above ambient. The adverse affect of high soil temperature was less severe with the later maturing varieties.

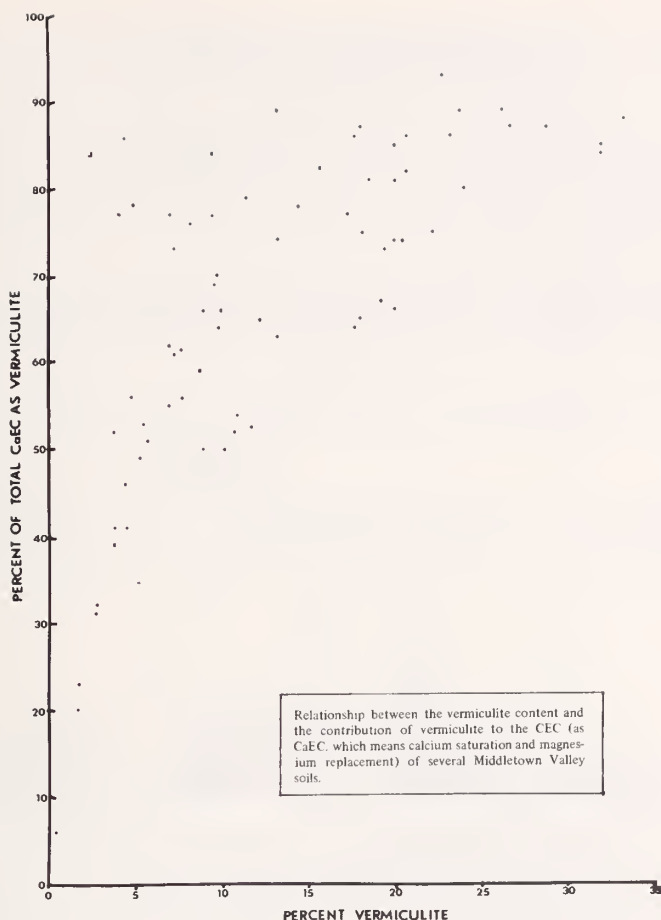
Project No. B-111



Soil heating maintained quality bermudagrass turf into the winter with air temperatures of 20 deg. F and below. Soil temperatures from top to bottom are ambient, 50 deg. F, 70 deg. F, and 90 deg. F. Bluegrass is on the left and bermudagrass is on the right.

CHARACTERIZATION OF THE CATION EXCHANGE COMPLEX OF MARYLAND SOILS

Studies have shown that the Fauquier and Myersville soils, developed from greenstone (chloritic metabasalt) in the Middletown Valley of Frederick County, Maryland, have very exceptional cation exchange capacity (CEC) properties. In contrast to Coastal Plain soils, where CEC is well correlated with clay content, the bulk of the reactive minerals in the Fauquier and Myersville soils occurs in the silt and sand fractions. This is especially true for subsoil horizons. But even for the surface horizons of these soils, it appears that about half of the CEC arising from inorganic materials is attributable to non-clay size fractions. It has also been shown that vermiculite is the main mineral responsible for the reactivity of the sand and silt fractions in these soils. This vermiculite apparently has formed by a mineralogical transformation from chlorite, the main mineral in the greenstone parent material of these soils.



Vermiculite has about the highest cation exchange capacity of any mineral known to occur in soils. It is capable of fixing potassium from potassium bearing solutions. A method for the quantitative determination of the amount of vermiculite in a material was developed based on the amount of potassium that is fixed. A rapid method was also developed for CEC determinations. Using these methods, it has been shown that B horizons of the Myersville and Fauquier soils have CEC values that range from about 25 to 50 milliequivalents per 100 grams. The B horizons of representative medium-textured Coastal Plain soils such as the Sassafras were found to have CEC values of less than 10 milliequivalents per 100 grams. Application of the techniques to particle size fractions of a Myersville soil showed that for a deep C horizon 65 percent of the vermiculite present in this material occurred in the sand, 32 percent in the silt and only 3 percent in the clay. In a corresponding A horizon (plow layer), 11 percent of the vermiculite present was sand-size, 47 percent was silt-size and 42 percent was clay-size. The B horizons were intermediate with the bulk of the vermiculite occurring in the silt fraction.

Project No. O-79

GROUND-WATER MEASUREMENTS IN SOILS

A major criterion determining the potential use or modification of soils is the depth of water table. Studies on the fluctuation of water tables in various Coastal Plain soils of Maryland have been in progress for seven years.

Figure 1 shows the results of a study of several silty soils on the Eastern Shore. As noted in this and other studies, the relative position of the water table during the year is directly related to the drainage class of the soils as delineated by soil scientists. For example, the somewhat poorly drained Bertie soil has the highest water table level, while the moderately well drained Mattapex has the lowest levels during the year. Water-table levels have also been shown to be highest in the spring as a result of the soil being recharged with water during the fall and winter. Evaporation and transpiration rates are low during these periods. However, data show that the rainfall distribution will dictate the water table fluctuation during a given year. In 1969 the water levels of all soils were low because of the limited precipitation.

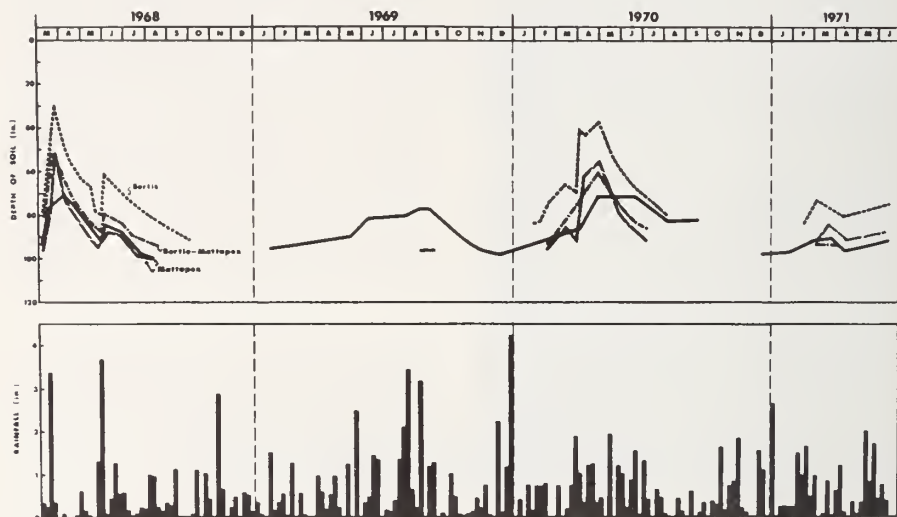


FIGURE 1 Ground water levels and precipitation at 4 sites, Wye Institute 1968 - 71

These studies have shown that the information in the soil survey can provide valuable information on predicting water table levels in landscapes of Maryland's Eastern Shore.

Project No. O-48

EFFECTIVENESS AND RESIDUAL PROPERTIES OF PREEMERGENCE TURF HERBICIDES

Seven commonly used preemergence turf herbicides in various formulations have been applied to Kentucky bluegrass - creeping red fescue turf for seven consecutive years. The purpose was to determine herbicide effectiveness and study the long-term effect on turfgrass quality. Bandane, Bensulide, DCPA, Tricalcium arsenate and granular formulations of Siduron and Benefin have consistently given excellent control of crabgrass. Repeated annual applications of Bandane have severely thinned the turf. Tricalcium arsenate injury to turf has been erratic with thinning of the turf and plant dwarfing occurring in some years. Granular materials have consistently provided longer crabgrass control than wettable powders and emulsifiable concentrations. Bensulide, DCPA and Siduron continue to provide excellent crabgrass control with maximum safety to turfgrass and minimum residual carryover from year to year. The continued use of environmentally stable chemicals such as Bandane and tricalcium arsenate can be detrimental to quality turf production.

Project No. B-107

BREEDING AND EVALUATION OF KENTUCKY BLUEGRASS FOR TURF

Sixty-four Kentucky bluegrass cultivars are being evaluated singly and in combination to determine their potential for providing quality turf under Maryland soil and climatic conditions. The initial plots were established in the fall of 1968 at the Hopkins Plant Research Farm near College Park and on the Eastern Shore near Centreville, Maryland. Cultivars performing extremely well under variable mowing heights and locations are Merion, Warrens A-34, Birka, Sodco, Fylking Warrens A-20, Pennstar, Vantage and Windsor. Cultivars performing poorly under proper lawn management were Delta, Nuggett, NJE P-5 and NJE P-56. Cultivars such as Vantage, Windsor and Warrens A-10 have exhibited extreme *Sclerotinia* Dollar Spot resistance. Cultivars noted for extreme *Helminthosporium* leafspot resistance were Warrens A-34, Pennstar, Warrens A-20 and a blend of Merion, Fylking and Pennstar. It appears that there is no single cultivar totally disease resistant and capable of providing the highest possible turf quality under all the variable conditions existent in the state of Maryland. Data from this experiment supports the recommendation for Kentucky bluegrass blends in Maryland.

The cultivars are also being evaluated for resistance to ozone air pollution. Newport, Sydsport, Merion, Fylking and Windsor are exhibiting above average resistance to air pollution.

Project No. B-109

MANAGEMENT REQUIREMENTS OF TUFCOTE BERMUDAGRASS

Recent increases in the popularity of Tufcote bermudagrass made it necessary to determine management procedures that would promote the highest quality bermudagrass turf in Maryland. The effect of various fertility practices, overseeding procedures and mowing heights were investigated to determine which combination of these management factors would provide the highest quality turf over the greatest period of time. Soluble sources of nitrogen such as ammonium nitrate and urea gave quick growth response, but maintenance of good color required monthly applications of one lb N/1000 sq. ft. Urea formaldehyde applied at nine lb N/1000 sq. ft. in April gave near equivalent color throughout the season. Overseeding with annual ryegrass in the fall at a rate of 14 lb/1000 sq. ft. provided the best winter green color and easiest spring transition back to bermudagrass. Mowing bermudagrass every two or three days produces the least amount of scalping and the highest quality turf.

Project No. B-104

QUALITY TURF PRODUCTION

Expanded urban population has created increased demand for information about management practices necessary for quality turf production on golf courses, sod farms, athletic fields and home lawns. Studies have recently been initiated; 1) To determine the traffic bearing potential and quality of zoysiagrass - bluegrass combinations for golf course tees. 2) To determine management practices necessary for fastest and highest quality production of 90 percent Tall Fescue - 10 percent Kentucky bluegrass sod. 3) To determine the mixtures and blends of cool season grasses that will provide the greatest traffic bearing potential under varying nitrogen fertility regimes and mowing heights and 4) To study the influence of herbicides and management factors on the eradication of weeds from quality turf. The experiments necessary to achieve these objectives are currently in various stages of installation and no data is available.

Project No. B-125

FORAGE CROP VARIETY EVALUATION

Twenty alfalfa varieties were seeded in April, 1969 at the Forage Research Farm, Clarksville, and tested for yield and persistence through 1972. The top producing varieties from '69 through '71 were: WL 305 (6.24 ton/A 3 yr. average), Team (6.04), Weevlchek (6.02), Saranac (5.96), WL 210 (5.96) and Iroquois (5.95).

In the summer of '71, severe wilt and root rotting disease depleted the Team stands. Top yielding varieties based on four-year averages were: WL 305 (6.69), Iroquois (6.06), Saranac (6.00), Weevlchek (5.99) and WL 210 (5.98). These varieties showed good persistence as did WL 306, Titan and Thor, but had lower average yields (5.74, 5.67 and 5.64 ton/A, respectively). Two commercial hybrid alfalfa stands were lost after one year in the study.

New alfalfa variety performance trials have been established at Clarksville and at the Wye Institute, Queenstown. Performance of commercially available varieties is being compared along with promising experimental lines.

Project No. B-77

RED CLOVER IMPROVEMENT

Improvement in red clover forage yields and stand persistence has been recognized in the recently released and recommended red clover variety, Kenstar. This variety was evaluated in Maryland for three years. It showed superior forage yields to those of Chesapeake and Pennscoct in tests conducted at Clarksville during 1968-1970. Stands of Kenstar persist six to eight months longer than those of Chesapeake.

Also under evaluation in newly established tests is the first true doublecross hybrid red clover. The hybrid is being compared to standard varieties Chesapeake, Kenstar, Pennscoct and common medium red clover. In preliminary trials, the hybrid red clover has shown excellent vigor, recovery and dry matter production.

Project No. B-76

BREEDING FOR IMPROVED FORAGE QUALITY

This project, aimed at improving the nutritive quality of several important forage species in the Northeastern United States, was initiated in 1971. The forage species being studied in Maryland include alfalfa and crownvetch.

Alfalfa contains protein of excellent quality. However, the quantity of protein within alfalfa plants varies greatly, and the amount present in alfalfa forage is affected by maturity and environmental factors.

These studies were initiated to increase the level of protein in new alfalfa strains and to improve the consistency of its presence in the forage materials. Simazine has been found to enhance protein synthesis in several species of plants. It is recommended as a dormant weed control chemical in alfalfa. We have exposed spaced planted alfalfa populations to Simazine at one lb./A and then selected for vigor and tolerance to simazine and then for protein content (%) and total protein/plant (% protein x dry weight of plant).

After two cycles of selection, average protein content has been increased from 20.8 to 24.7 percent. Total protein per plant has ranged from 3.0 to 7.0 grams per plant in the selected population, indicating genetic variability still exists for further improvement.

An added bonus has been found in these studies. We have found that higher protein content is a good indicator of higher dry matter digestibility. Therefore, we have improved the nutritive value and utilization by selecting for high protein levels in alfalfa.

Project No. B-120

RESIDUAL EFFECT OF THIRTEEN CROPPING SYSTEMS ON CORN YIELD AND SOIL AGGREGATION

This experiment is just starting and no yield data have been collected. It is designed to study the length of time that soil aggregation will persist in the soil under continuous corn. Eighty-one plots on Beltsville silt loam that have been in thirteen cropping systems during the past 20 years will be put in continuous corn. Soil samples will be taken each year in July for organic matter and soil aggregation determinations. Measurements were made in July of 1972 to serve as base measurements for comparison with measurements in later years under continuous corn. Organic matter ranged from a low of .71 percent in plots that have been in bare fallow to 2.24 percent for the plots that have been in continuous grass. Large differences in soil aggregation are also present. Two levels of nitrogen (100 lbs. and 170 lbs. per acre). will be used on the corn.

Project No. O-81

EFFECT OF FALL AND SPRING PLOWING ON SOIL MOISTURE, SOIL AGGREGATE STABILITY AND CORN YIELD UNDER VARIOUS CROPPING

Six years of fall and spring plowing of land under various cropping systems ranging in intensity of cultivation from continuous cropping of corn and soybeans to a five year rotation of corn, wheat and three years of orchard grass - ladino clover mixture have been completed in this project. Fall - and spring-plowed corn plots have yielded about the same (112 bu average for fall and 109 bu average for spring). Fall plowing was considerably better on two of the six years, while the other years showed equal or superior yields for the spring-plowed plots. The average soybean yield for fall-plowed plots was 37.3, while the spring-plowed plots produced 38.6 bu. Spring-plowed plots had slightly better tilth as indicated by the soil aggregate stability measurements. Large differences were found in soil aggregate stability between cropping systems with continuous soybean plots being the lowest and the plots in corn, wheat, three years of orchardgrass - ladino clover mixture being the highest. Continuous corn yielded 92 bu per acre, while rotation corn with one or more years of hay or grass crops in the rotation yielded 123 bu per acre. Continuous soybeans produced 35 bu per acre, while soybeans in a rotation with corn and wheat produced 40 bu per acre.

Project No. O-76

THE USE OF 2-CHLOROETHYLPHOSPHONIC ACID TO IMPROVE RIPENING RATE AND MATURITY OF MARYLAND TOBACCO

The objective of this experiment was to compare rates of 2-chloroethylphosphonic acid and the effect of leaf maturity at time of application upon yield, price and acre values of cured tobacco.

The 2-chloroethylphosphonic acid caused the top leaves to turn a definite yellow color similar to ripeness within four days of application. This chemical also caused premature "firing" and loss of bottom leaves at a rapid rate. It was necessary to harvest within three to four days after the application of the material.

Curing was faster where the plant was treated. The yellowing stage was marked by a complete yellowing of the top leaves in less than four days after harvest. All the leaves had turned a red-brown color within only two weeks after harvesting, however the midribs of the leaves were not dried in this period.

The material was more effective if applied 10 days or more after topping, because of the greater leaf maturity at this point. The price per pound of the cured leaf was about four cents higher for this treatment than when applied at time of topping because of better maturity. The acre value was up to \$300 higher when the combination of chemical and full maturity resulted in complete yellowing and optimum maturity. This occurred when the chemical was applied at least 10 days after topping and harvesting was over 15 days after topping.

Different rates of 2-chloroethylphosphonic acid showed no significant differences as the rates resulted in the same effect on yield, price and acre values.

Project No. B-101

DEVELOPMENT OF TECHNIQUES FOR PRODUCING TOBACCO SEEDLINGS IN PRESSED PEAT-SOIL CUBES

Tobacco seedlings grown in pressed peat-soil cubes were planted with a 2-row commercially available peat pot mechanical transplanter. These experimental seedlings were compared to seedlings specially selected for uniform size and shape from a conventional plant bed and to seedlings which were irregular in size and shape as drawn from a conventional plant bed. Each of these three treatments was planted with water and without water applied to the root system at transplanting.

Data were recorded approximately three weeks after transplanting on early plant growth, uniformity, size and vigor for each treatment. This was expressed as an index from 0 to 10 with a 10 rating being the highest in uniformity, size and vigor. Differences were quite pronounced in seasons where the transplanting season and period following was dry and hot and considerable moisture stress resulted. The addition of water improved the seedling's performance for all three treatments. The seedlings in cubes were superior to the other transplants during these dry periods and acceptable for standard use even without the use of water. The performance of other



Uniform seedlings grown in pressed peat-soil cubes above provided over 32,000 transplants per 100 sq. yds.

seedlings tested was not acceptable without the use of water. Thus, under conditions of stress the cubed seedling exhibited more uniform and vigorous early growth.

Results from cured leaf studies indicated that under moisture stress conditions, as in the 1969 planting period, the cube transplant was superior to the control in yield and acre value. The yield where cube transplants were used was 13.8 percent larger than the control and acre value was 16.7 percent greater.

However, tobacco yield, acre value and price were the same for all treatments in seasons of average-or-better soil moisture. Thus, the cubed seedlings were agronomically feasible. Measurements of uniformity indicated that the specially selected seedlings and the cubed seedlings were superior in uniformity to the other treatments. This was true regardless of the soil moisture levels from year to year.

Project No. B-118

PHYSIOLOGICAL RELATIONSHIPS OF TOBACCO TO CULTURAL, FERTILIZATION, CURING AND FERMENTATION PRACTICES

The physiology of Maryland type tobacco is greatly affected by environmental factors such as the moisture regime and cultural practices such as nitrogen fertilization, topping time and topping height. Major changes in the chemistry of the leaves which influence the quality of the cured product include the total nitrogen levels and total alkaloid levels. Excessive amounts of rainfall or irrigation during the growing season tends to lower both the total nitrogen and total alkaloid levels. Unusually dry conditions tend to induce higher levels of nitrogen and alkaloids than desired. Increasing the rate of nitrogen fertilization generally increases the alkaloid levels along with increasing the total nitrogen levels.

Since alkaloids are produced in the roots of the plant and are transported to the leaves where they accumulate, the levels of alkaloids are greatly influenced by the time of topping and the plant height. Plants topped at 13 leaves in height prior to flowering have much higher alkaloid levels (2.80 percent) than those topped after flowers begin to form (1.39 percent) in the bud. Topping time tends to have less influence on total alkaloid levels in plants topped at 18 leaves (2.00 percent vs. 2.48 percent for preflowering vs. bud stage topping). Thus, to minimize the buildup of alkaloids, a later topping time is recommended if a lower topping height is employed.

The type of alkaloids present in cured tobacco is of equal importance as their total quantity. The presence of appreciable quantities of nornicotine gives the tobacco an undesirable taste when smoked. The genetic makeup of the plant determines the type and quantity of alkaloids the plant produces. All breeding lines are screened each year for the presence of nornicotine and discarded if appreciable quantities appear. At present, all established varieties available to growers have very low levels of nornicotine.

Project No. B-89

FACTORS RELATED TO IRRIGATION OF TOBACCO

Supplemental irrigation has been shown to influence both the chemical and agronomic characteristics of Maryland type tobacco. Chemical characteristics such as total nitrogen and total alkaloid levels are closely related to the moisture regime during the growing season. Agronomic characteristics such as yields, average price and value per acre are likewise closely related to soil moisture.

Studies conducted at the Tobacco Experimental Farm near Upper Marlboro, Md. over the past four years were designed to provide supplemental irrigation only when rainfall amounts or distribution became inadequate. As a consequence, comparisons with the non-irrigated tobacco generally vary from year to year depending on the rainfall patterns. Irrigation has its maximum influence and benefit during years with inadequate amounts or poor distribution of rainfall.

Irrigated tobacco generally shows lower total nitrogen levels and lower total alkaloid levels compared to non-irrigated tobacco. Results from 1969 show irrigated tobacco had an average alkaloid level of 1.26 percent compared to 1.61 percent for non-irrigated tobacco. Lower alkaloid levels are generally more desirable than higher alkaloids. The lower alkaloid levels in the irrigated tobacco may be related to its earlier maturity and harvest, since less time is available for alkaloid synthesis and accumulation.

During years with less than adequate rainfall, irrigation resulted in higher yields, better quality as indicated by higher average price per pound and higher dollar return. It is also recognized that significant differences in response may exist among the various Maryland varieties. Increased yields with irrigation may occur for certain varieties such as Md. 64. Improved quality may be the response for other varieties such as Md. 609 compared with standard varieties such as Catterton. However, both responses can result in higher value per acre.

In studies comparing the influence of nitrogen application (70 vs. 90 lbs/A) with and without irrigation, higher yields and value were generally observed in the tobacco receiving both irrigation and higher nitrogen applications. Some danger may exist from irrigating tobacco with lower nitrogen applications. The higher nitrogen applications generally increased both the total nitrogen levels and total alkaloid levels in the cured product.

Project No. B-99

PHYSIOLOGICAL RELATIONSHIPS OF TOBACCO TO ENVIRONMENTAL, CULTURAL AND GENETIC FACTORS

Tobacco as a species has been found to be highly sensitive to air pollution, particularly to pollutants such as ozone and SO₂ which are commonly found at damaging concentrations in the air in the Washington-Baltimore area. Several factors such as moisture regime, nitrogen fertilization and varieties have a great influence on the response of plants to air pollution.

Field and greenhouse studies are currently underway to gain information on the response of Maryland tobacco varieties to ozone and ambient oxidants. In addition to cultural practices, major emphasis is being placed on factors associated with resistance mechanisms and levels of resistance in varieties. Varieties such as Catterton and Md. 59 appear to have a low level of resistance to ozone. Wilson and Gertz appear to have a moderate level of resistance at oxidant concentrations which currently exist. Varieties such as Md. 609, Md. 64 and Md. 10 appear to have slightly more resistance to ozone than Md. 59. Crosses between varieties with different levels of resistance produce hybrids with intermediate levels of resistance to that of the two parents, indicating the level of resistance can be manipulated through breeding. It appears that the level of resistance in the Wilson variety is sufficient to serve as a source of resistance for breeding work to improve the level of resistance in our more popular varieties such as Md. 609.

Project No. B-122

THE BIOLOGICAL ACTIVITY AND MODE OF ACTION OF HERBICIDES USED ON CORN, SOYBEANS AND TOBACCO

Field research using herbicides for the control of weeds was conducted in corn, soybeans and tobacco at five locations within the state. Yellow nuts-edge control in conventional planted corn tillage was best with butylate, atrazine and alachlor in a three year study. Combinations of atrazine + butylate preplant incorporated resulted in 75 to 82 percent control. Postemergence applications with atrazine at 2 lb/A + 1 gal/A of oil resulted in 67 percent control which was 10 percent better than the same treatment without oil. Combinations of butylate preplant incorporated + atrazine postemergence with oil resulted in 94 percent control. The same combination without oil resulted in 73 percent control. Atrazine, 2 lb/A preplant incorporated, + atrazine, 1 lb/A postemergence with oil, resulted in 80 percent control. In other work conducted in conventional tillage corn, two antidotes for EPTC and butylate injury to corn were evaluated and proved successful. These antidotes were 0.125 percent R-25788 and 0.5 percent 1,8 naphthalic anhydride; when these materials were applied as seed treatments, corn injury was prevented at 16 lb/A butylate and 9 lb/A EPTC. R-25788 was also effective when incorporated in the soil as a tank mix with EPTC or butylate.

Several new triazine herbicides, cyprazine and cyanazine, were evaluated and found to be as effective as atrazine preemergence, but causing some corn injury, especially when applied late postemergence to six leaf stage corn or beyond.

In no-tillage corn, fall panicum control was best with simazine alone or in combination with other weeds.

In soybeans, fluorodifen was found to exhibit postemergence activity on broadleaved weeds, but was injurious to soybeans that were beyond the crook stage. In no-tillage soybeans following barley, paraquat was found to be needed as a foliar spray to kill existing weeds and the residual herbicides linuron + alachlor resulted in adequate season-long control when soybeans were planted in 20 inch rows.

In tobacco, the following herbicides have been tested for the past four years and found to be effective on crabgrass and foxtail: pebulate, diphenamid, benefin and combinations of pebulate + R-7465.

Project No. B-117

USE OF HERBICIDES TO CONTROL WEEDS IN FORAGES

Residual herbicides are being applied yearly to dormant, established alfalfa to determine any deleterious effects on alfalfa stand and weed populations. After one year, alfalfa stand and vigor was severely injured by several herbicide treatments, notably simazine at 4 lb/A, GS 14254 at 8 lb/A and BAY 94337 (Sencor) at 4 lb/A. Alfalfa injury was observed with diuron (Karmax) (4 lb/A), GS 14254 (4 lb/A), terbacil (Sinbar) (0.8 and 1.6 lb/A) and BAY 94337 (Sencor) (1 and 2 lb/A), but there was no significant alfalfa stand loss.

Dandelions in established alfalfa are an increasing problem. Several rates of terbacil and Amchem 68-72 were applied to dormant, established alfalfa for the control of established dandelions. At the high rates, both herbicides caused excessive alfalfa injury when evaluated one month after application. When evaluated in October, alfalfa stand reduction was about 50 percent for 2.0 lb/A of Amchem 68-72 and 10 percent for 1.5 lb/A of terbacil. Early dandelion control was good only at the 1.5 lb/A rate of Amchem 68-72 and poor at 1.0 lb/A, but when observed in October the 1.0 lb/A rate improved to 60 percent and 1.5 lb/A to 90 percent. Terbacil at 1.0 lb/A resulted in fair dandelion control when observed early and late; terbacil at 1.5 lb/A resulted in 95 percent dandelion control.

Project No. B-79

BREEDING FOR DROUGHT TOLERANT CORN HYBRIDS

Response of six corn hybrids to irrigation was studied at each of three planting dates on a Mattapex silt loam soil in 1969, 1970 and 1971 at the Wye Institute Farm. In general, the yield of hybrids in irrigated treatments were higher at the earliest planting date (late April) and lower where planting was delayed. In non-irrigated treatments, the early planting date did not always show highest yields, but best yields resulted when moisture was adequate during the grain forming period. Irrigation increased yield in many cases, but in some instances decreased yield because of too much moisture as a result of heavy rains following addition of supplemental water. It is probable that consistently high yield increases with irrigation will not result every year on soils of this nature in Maryland.

Measurement of drought tolerance in these hybrids was inconsistent due to uncontrolled environmental conditions.

Project No. B-112

VARIETAL IMPROVEMENT IN BARLEY AND WHEAT

Uniform nurseries and variety tests of breeding stock from various states have revealed several promising barley and wheat varieties adapted to Maryland. In barley, Tenn 65-117 has been the most promising strain over the period 1969-1972. It has average 83.5 bushels per acre as compared to 65.3, 66.1 and 61.2 bushels for Rapidan, Hanover and Knob, respectively.

Barley strains with malting potential were evaluated as a potential replacement to Tschermak, the current malting variety grown in Maryland. New York selection 6005-18, a six-rowed variety, showed the most promise.

New wheat releases which have performed well in the state include the early maturing varieties Arthur, Arthur '71, Abe and Coker 68-15.

Two wheat varieties were exposed to ozone at anthesis and subsequent effects on yield and yield characteristics were examined. Lower yield in ozone treated plants could be accounted for by reductions in kernel weight, seed per plant, seed per head and percent seed set.

Fertilization studies with stiff-strawed wheats revealed that topdressing in the spring with up to 90 lbs. of nitrogen would be profitable.

Project No. B-116

BREEDING OF IMPROVED VARIETIES OF FORAGE SPECIES ADAPTED TO THE NORTHEAST

Alfalfa is frequently referred to as the queen of the forages and rightfully so, since it is an excellent feed for livestock. In Maryland, successful alfalfa production has been plagued by serious insect problems like the alfalfa weevil and many diseases including anthracnose, leafspots and root and crown rots. Alfalfa improvement in this project has centered upon the development of adapted alfalfa varieties with multiple pest resistance. This work has been closely coordinated with USDA scientists.

The first alfalfa variety derived from this project was Team, which was released in 1969. Team has good levels of resistance to the alfalfa weevil, common leafspot organisms and some resistance to anthracnose. It produces high yields which are equal to those of Williamsburg, Saranac and Vernal. Higher levels of resistance to anthracnose and bacterial wilt have been added to Team - related germplasm during 1969-1972. This germplasm has been coded as MSHpFAn₄W₄. In tests conducted in 1971 and 1972 at the Wye Institute, MSHp6FAn₄W₄ has produced yields of 7.16 and 7.36 tons of forage per acre as compared to yields of 6.54 and 6.24 for Williamsburg, a commonly grown alfalfa variety in Maryland. The built-in resistance to the many pests attacking alfalfa has given MSHp6FAn₄W₄ superior survival and vigor over non-resistant or partially resistant varieties.

Screening for higher tolerance to Sclerotinia crown and stem rot in alfalfa has been advanced to the second cycle of selection in the laboratory. The third cycle of selection will be made from a field planting of 1,200 individual alfalfa clones made at the Wye Institute.

Project No. B-56-1

SOYBEAN VARIETAL IMPROVEMENT

Research findings of studies aimed at developing improved soybean varieties for Maryland farmers have resulted in the release of four new soybean varieties and the advancement of many potentially outstanding breeding lines. The varieties that were released and their year of release were Williams (1971), Wye (1971), Columbus (1971) and Essex (1972). The Wye variety was the first soybean variety developed and released by the Maryland Agricultural Experiment Station in cooperation with the USDA. These new varieties will occupy most of Maryland's soybean acreage by 1975.

Breeding for resistance to the Mexican bean beetle, the most serious insect pest of soybeans, has progressed to the point where a resistant line (V69-156) will be released in 1973-74. This line has shown good yielding and seed quality characteristics, while preventing yield loss to Mexican bean beetle injury because of innate factors. V69-156 is a late-maturing line that will be adapted to the lower Eastern Shore where the beetle is most damaging. Further breeding progress is being made to develop earlier varieties of soybeans with beetle resistance to combat the northern advancement of the beetle in Maryland. In addition to Mexican bean beetle research, resistance to the corn earworm and soybean looper are being incorporated into lines adapted to Maryland.

Progress towards a new group of soybean varieties with responsiveness to fertilizer has been steady over the last four years. During the first years of this study, genotypes that show a unique response to potassium were isolated. This characteristic has been transferred to adapted soybean varieties through a series of intermatings. Yield trials in low, medium and high fertility soils will be conducted in 1973. The objective of these studies is to select those lines of soybeans that produce a more economic return when grown under good to excellent soil fertility. Soybean varieties now being grown have not shown a significant yield response to good fertility programs.

The use of a winter nursery in Puerto Rico has greatly enhanced the soybean breeding program. By utilizing the winter nursery, where two crops can be grown each winter, the time from making the initial cross until variety release has been reduced from 10-12 to 5-7 years.

Work on improving soybean yields in the popular double-cropping system of small-grains and soybeans has revealed the importance of the following facts:

- (1) Planting soybeans in narrow row widths results in yields 5-20 percent higher than in 30" rows.
- (2) Planting as early as possible using early small grain varieties and no-tillage planting method produces highest yields.
- (3) Planting York, Columbus and Essex soybean varieties increases the potential of higher yield.

Project No. B-43

PROJECT REPORTS

ANIMAL SCIENCE

ANIMAL SCIENCE

The Department of Animal Science conducts research in the areas of breeding, management, nutrition and physiology. The primary objective of these research projects is to improve the economic and esthetic usefulness of beef cattle, horses, sheep and swine. This objective dictates that research efforts should be oriented to promote the highest quality of living within the limitations of environmental resources, while holding high priorities for environmental enhancement. Consumer tastes and desires for leaner red meats have been changing and the increased use of animals for recreation and biomedical research has given a new dimension to research in Animal Science in recent years. Livestock producers are constantly applying new information to produce animals that efficiently provide products that conform to the requirements of our modern society. The Department's research efforts are directed toward producing a more desirable, minimally priced product at a level of efficiency which provides profit opportunity for the livestock producers.

EFFECTS OF ROUGHAGE PREPARATION

The production of vegetable crops for human consumption characteristically involves the production of large quantities of plant materials that are not directly consumable by man. In many cases these inedible portions of the crops are accumulated as waste products of processing plants, in other cases they are left at the site of harvest. The possibility of using such waste products as animal feeds has long been recognized. Products such as grain processing by-products, corn canning wastes and citrus processing by-products are the basis for sizable feed processing or livestock feeding enterprises. However, many vegetable wastes are high moisture, perishable products which are frequently uneconomical to collect, transport and store. Others are low-protein, high-fiber materials which are of limited use as animal feeds unless they are properly supplemented with protein and energy rich feeds. Experience with hay crop silages has shown that young high moisture, high protein forages frequently make poor quality silages. However, the quality of the silage can be improved by mixing the wet forage with dry low-protein materials. This requires protein supplementation which lowers moisture content of the ensiled mass. The mixing results in a feed product superior to either high moisture silage or the dry material if either was fed alone.

In experiments designed to determine the chemical composition and palatability of tomato waste materials as criteria of the usefulness of these products as feed for sheep, it was found that tomato vine dry matter frequently contained high levels of insoluble ash, presumably due to external contamination with sand or soil. Apparent digestibilities by mature wethers were determined for tomato vines that had been untreated, washed, or to which sand had been added prior to ensiling. Apparent digestibilities were also measured for tomato vines which received the above treatments, but were also sprayed with pesticides (Maneb and Sevin) either one, three or seven days prior to harvesting and ensiling. Washing of the vines to remove sand did not appreciably affect the apparent digestibility of crude protein (79.6 percent for washed tomato vines versus 79.5 percent of the untreated tomato vines). The apparent digestibilities of total dry matter, tomato vine dry matter and tomato vine ash were all significantly lower for the untreated tomato vines than for the washed tomato vines. Tomato vine dry matter digestibility ranged from 62 to 67 percent. The addition of sand to the tomato vine silage tended to reduce the digestibility of dry matter, crude protein and ash. Results of digestion trials conducted to measure the effects of pesticides upon digestibilities indicate that digestibility of dry matter, crude protein and ash harvested one day after spraying the vines were lower than those for vines harvested prior or subsequent to spray application. Thus, the depression in digestibility is apparently due to the spray application rather than to the age of the tomato vines. These trials also showed that digestibilities seven days after spraying were much higher than those observed one and three days post spraying. Two digestion trials were conducted to compare digestibility of direct-cut tomato vine silage and dried tomato vines. Dry matter, crude protein and ash were more digestible in the direct-cut tomato vines than in the dried tomato vines.

Studies on the feasibility of using organic acids or preservatives for high moisture forages have also been initiated. Alfalfa at approximately 35 percent moisture was sprayed in the windrow with propionic or acetic acids in quantities equivalent to 1.5 percent of dry matter. The hay was then baled and stored in open loft storage. Snapped ear corn at 35-40 percent moisture was ground and mixed with urea (1.5 percent of total weight) and propionic or acetic acids (3 percent of total weight) and stored in burlap bags. Control lots (w/o organic acid) of both materials were stored. Maximum temperatures in the snapped corn treatments were reached after five days in storage and average 16.1, 18.2 and 50.2 degrees C in the propionic acid, acetic acid and control lots, respectively. Temperatures in the control lots remained above 40 degrees C after 28 days in storage while the acid treated lot had temperatures of 11-13 degrees C at this point. Studies to determine the preservation of nitrogen and dry matter, the acceptability of the feeds to sheep and the digestibility of dry matter and crude protein *in vivo* and *in vitro* are being conducted.

Project No. C-25A

PHOSPHORUS REQUIREMENTS AND SOURCES FOR EARLY-WEANED PIGS

The phosphorus requirements for baby pigs weaned at three weeks of age have not been definitely determined. Dramatic changes in commercial swine management accompanied by rapid genetic improvement of the general swine population has prompted the re-evaluation of the requirements for several nutrients. Many commercial producers are weaning pigs as early as three weeks following birth to improve the rate of reproduction in the sow herd and to make better use of confinement facilities. Economical diets rely on a high level of plant sources of nutrients when compared to the average diet of the suckling pig at the same age. It is known that phosphorus contained in plant sources is not as readily digested and absorbed as that obtained from animal sources such as milk or fish meal. The genetic improvement of swine has resulted in more rapid early growth. During early growth, bone tissue is being formed at a rapid rate. Phosphorus makes up a significant amount of the mineral deposited in bone growth. These considerations stimulated the study of the phosphorus requirement for optimum growth of weanling pigs between the ages of three and eight weeks, and an evaluation of the availability of phosphorus from menhaden fish meal.

Previous pilot trials had indicated that 0.8 percent calcium would be the optimum level in the diets for pigs of this age. Experimental levels of dietary phosphorus in the series of 0.2 percent, 0.3 percent, 0.4 percent, 0.5 percent, 0.6 percent, 0.7 percent and 0.8 percent were studied. Many factors were measured which might be influenced by the dietary level of phosphorus intake. These included rate of gain, feed consumption, feed efficiency and levels of phosphorus and calcium found in the following body tissues: intestines, liver, kidney, blood and bone.

The evaluation of all factors considered indicated that the dietary level of 0.6 percent of available phosphorus in combination with 0.8 percent calcium would support optimum growth, feed efficiency and feed consumption. A slight improvement was noted at the highest phosphorus level. The tissue analyses and growth and feed responses suggest that diets containing 0.8 percent phosphorus and 0.8 percent calcium will support maximum growth and feed efficiency in early-weaned pigs. However, it is doubtful that the additional response can be consistent enough and large enough to justify the cost of additional dietary phosphorus.

The results of this study also indicates that 100 percent of the phosphorus from menhaden fish meal is nutritionally available to the young weanling pig. This is in contrast to only 30 percent to 40 percent phosphorus availability from plant sources such as corn and soybean oil meal.

Project No. C-33

VARIABILITY OF OVULATION RATE IN SWINE

Swine producers are very dependent upon reproductive efficiency in their sow herd for the economic production of pork. Accurate timing in the management of breeding and appropriate management procedures to insure regular production of optimum-sized litters are fundamental to an economic swine producing unit.

The development of better recommendations for managing the sow herd has been hampered by the inability of reproductive physiologists to measure normal and abnormal levels of hormones which control the female processes of reproduction. Previously used analytical techniques have not been sensitive enough to measure the extremely low levels of hormones which control the normal reproductive function, or contribute to abnormal reproductive function.

The research in this area has developed a sensitive, precise and specific technique for measuring levels of luteinizing hormone (LH) circulating in the blood of a reproductively mature female swine. LH is the hormone which has a dominant role in regulating the reproductive cycle and maintaining pregnancy, once pregnancy has been established. The technique involves the use of radioactive tracers and antigen-antibody reactions. Thus, it is described as a radioimmunoassay.

This technique has been used to describe the levels and fluctuations of circulating LH in pregnant, non-pregnant and immature Yorkshire female swine. This information will be necessary to interpret the effects of hormone treatments which may be employed to control reproductive cycles and ovarian activity within a cycle. Studies involving hormone injections (estrogens and progestins) have shown that any treatment which changes ovarian function will be moderated and/or modified by the feedback mechanism which is implemented through LH.

Two other hormones, follicle stimulating hormone (FSH) and prolactin, are also involved in the regulation of ovarian function. Work to develop radioimmunoassays for these hormones has been completed. These new methods have been employed to describe the hormonal events, with new and useful precision, which are associated with normal reproductive cycles. A new statistical method has also been developed which permits much better interpretation of the hormonal activities that are present in the normal reproductive cycle. The capability of accurate and precise measurement of circulating levels of all hormones that control reproduction in swine is an accomplishment not achieved by any other research center.

The approaches and minimal successes in synchronizing estrus (heat) and optimizing litter size in swine have been based on incomplete information. The work reported here could bring us closer to achieving these objectives on the basis of sound principles.

Project No. C-34

CRITICAL EVALUATION OF PROTEIN SOURCES FOR YOUNG PIGS

The critical evaluation of protein sources for young pigs has been studied by two approaches: 1) The use of semi-purified diets with detailed measurement of pig responses and 2) Carcass evaluation of market hogs following the feeding of various diets after weaning.

With the first approach, pigs weaned at ten days of age were fed diets containing various combinations of defatted fish meal, dried skim milk and soybean oil as the protein sources. Young pigs cannot efficiently utilize soybean oil meal as the sole source of protein. Dried skim milk is an excellent but expensive source. The objective of the study was to determine a satisfactory level of substitution of the less expensive fish meal for the dried skim milk. Fish meal was included at levels of 0, 6, 12, 18, 24, and 30 percent of the total diet. The remaining protein portions of the diets were supplied by equal amounts of protein from soybean oil meal and dried skim milk. The protein content of all diets was 25 percent.

Fish meal at dietary levels of 6 and 12 percent and possibly 18 percent supported more growth and feed consumption until four weeks of age than did soybean oil meal. At higher levels (24 and 30 percent) feed consumption and growth were depressed. After four weeks of age, soybean oil meal was a superior source of protein (amino acids) for these early weaned pigs.

The observations on growth and feed consumption were augmented by determinations of blood serum levels of circulating amino acids. These determinations gave evidence that fish meal overcomes a methionine deficiency of the soybean meal up to four weeks of age. These determinations also indicated that the higher levels of fish meal contribute to an amino acid imbalance that is accompanied by a deficiency of threonine, one of the essential amino acids.

This study demonstrated that a defatted fish meal can be substituted for dried skim milk in diets for pigs up to four weeks of age and supplement soybean oil meal in a manner that supports efficient growth. The series of levels studied provides the necessary data to make economic decisions regarding protein sources for pigs weaned at an early age.

The second approach, i.e. carcass evaluation of hogs fed diets containing fish meal at an early age, demonstrated that 4 to 6 percent fish meal in the diets of pigs at an early age results in slightly improved carcasses. Feeding levels of fish meal to young pigs in excess of 16 percent results in improved carcasses, but the improvement is not sufficient to compensate for reductions in growth rate and feed efficiency that are the consequence of feeding fish meal at the higher levels.

Project No. C-35

ADEQUATE NUTRITION FOR PIGS WEANED VERY EARLY

Increased specialization and technical competence in the swine industry stimulates the search for additional ways to maximize overall efficiency in pork production. Reduction of practicable weaning age, from the conventional eight weeks, to three to five weeks has provided for economies in feed costs and much more flexibility in the management of the sow herd. By following early weaning practices, many producers can now produce five litters of pigs in a 24 month period, rather than the four litters which are possible by conventional practices.

Pigs could, theoretically, be weaned at an even earlier age than three weeks. The nutrient requirements are fairly well established and management procedures which support performance comparable to suckling pigs have been developed. However, the successful procedures appear to be limited by unfavorable economics and the necessity of feeding a liquid diet to the very early weaned pigs. Research has been conducted to study the feasibility of feeding dry meal diets composed of relatively economical feedstuffs to pigs weaned at seven days of age.

The use of a substantial amount of dried skim milk in dry diets for seven day-old pigs produced satisfactory gains and feed efficiency. Additional sugar in the form of glucose supported additionally favorable gains. The weight and feed efficiency of pigs fed in this manner compared favorably, both in terms of economy and level of performance, with pigs weaned at three weeks of age. These comparisons were made when pigs from both ages of weaning reached eight weeks of age.

Partial substitutions of menhaden fish meal and a defatted menhaden fish meal were made for the dried skim milk component of the experimental formula. The fish meals, on the basis of chemical composition and nutrient content, should substitute for the more costly dried skim milk, and thus achieve greater economy. The performance of pigs fed the diet with a partial substitution of defatted menhaden fish meal did approach that of the pigs fed the diet containing the dried skim milk. Although their growth and feed efficiency were slightly lower, the economy of this diet formulation was the best of those studied. The substitution of menhaden fish meal without the fat removed was not satisfactory. Pigs fed this diet gained slowly and inefficiently. Poor feed consumption was also observed.

Project No. C-38

ANALYSIS OF RECORDS OF BEEF CATTLE HERDS IN MARYLAND

Since the 82nd, Annual Report held 1968-69, a number of major developments have occurred relative to this project. Analyses of the data mentioned in that report were temporarily terminated because of a change in personnel, and later permanently terminated because of the request to cease gathering data on the project June 30, 1971. Analyses based on the total data have been initiated.

The data include those on 952 calves born during the years of 1963 through June, 1971. Totals of 340 male and 287 females were measured at 225 days of age. Of the latter, 272 completed individual feeding trials and were measured at that time: 23 at 309 days of age, 211 at 337 days of age and 38 when 365 days old. Of these females, 135 were measured at two years of age and four when five years old. Thirty-six males were measured at two years of age and four when five years old. All calves were weighed at birth and approximately 14-day intervals until weaning at 225 days of age. During individual feeding trials, weights were obtained each 28 days. Consumed feed was also recorded on 28-day intervals.

A number of different studies on the complete data have been initiated; some are now nearing completion. A companion study to Project No. C-45 involves the dimensional differences between bulls weighing in the ranges of 900-950 pounds and 1,000-1,050 pounds. In general, increases in length in the region of the rib were not statistically significant, but other length measurements in the region of the body and hindquarter were significant, primarily at the 1 percent level. Measurements of the depth were significant at the 1 percent level with the exception of some segments of the rear quarter. Widths along the back, loin, hooks and rump, as well as heartgirth, paunchgirth and rear flankgirth were all significant at the one percent level. These increases were not due to the depositions of much greater depths of fat as these were all statistically insignificant. In another study, dimensional differences from one age to the next were compared. With few exceptions, the females grew from 10 percent to 20 percent in length and width measurements from 225-337 days of age. The males usually grew 10 percent to 20 percent in length from the shoulder point to paunch and in the rear legs as well as in most depth measurements from 225 to 365 days. However, they grew 20 percent to 30 percent in depth along the back from the 12/13 rib rearward. From one to two years of age, the males and females followed a similar pattern with growth in the regions of the wholesale loin and some parts of the round being from 0 percent to 10 percent. The rest of the body grew from 10 percent to 20 percent. The males continued to increase in width at a greater rate than the females.

Studies on the partitioning of consumed TDN into that associated with estimated requirements for maintenance and gain by the use of several different equations have indicated that maintenance and gain together account for about one-half to two-thirds of the TDN consumed by bulls and somewhat less by females. The formula of Lofgreen and Garrett, as well as those in the 1970 edition of "Nutrient Requirements of Beef Cattle" (Natl. Acad. Sci.), did not fit the data collected under this project for, apparently, a number of reasons. Further study will be made in this area.

Heritability studies made to date indicate that heritability is sufficiently high to warrant the suggestion that breeders of beef cattle could increase the net profit of the industry by feeding animals individually and using the results on economy of gain in selection programs.

All of the above work has been done in cooperation with Wye Plantation, Queenstown, Maryland, and Dr. J. L. Carmon, Director, Computer Center, University of Georgia. Computer time has been furnished by the Computer Science Center, University of Maryland.

Project No. C-39

PROCUREMENT AND STUDY OF THE GNOTOBIOTIC RUMINANT

The nutrition and physiology of ruminant animals is distinguished by the complex symbiotic relationship with the bacteria and protozoa that normally inhabit the rumen. Germfree or gnotobiotic technology has enabled researchers to initiate studies on these animals without the influences and complications arising from microbial populations. Lambs were delivered by hysterectomy and reared in sterile, flexible plastic isolators. Control, lambs born naturally, were removed from their mothers and reared in individual cages. All lambs were fed a sterile synthetic milk (SPF-lac) for six weeks. A baby pig diet was made available to all lambs throughout the 10 week experimental period. While both control and experimental lambs readily drank large amounts of SPF-lac, the control animals, with one exception, ate greater quantities of the dry sterilized diet. This was reflected in the generally greater size and better condition of the control animals.

Both groups of lambs exhibited near normal blood glucose levels at one week of age. The gnotobiotic lambs had higher blood glucose concentrations than controls during most of the experiment. The preweaning decrease in blood glucose levels in both conventional and gnotobiotic lambs was most likely caused by the loss of fetal red blood cells. However, the subsequent drop in glucose concentration in the controls during the weaning period was indicative of the adjustment in energy metabolism from an enzyme system utilizing glucose to a fermentation system producing VFA. The gnotobiotic lambs displayed a relatively stable blood glucose concentration during weaning. However, when no milk was available to them, their blood glucose levels steadily declined to a concentration less than that found in the controls. This decline was probably caused by a suboptimal level of nutrition rather than an inherent ability of the gnotobiotic lambs to lower blood glucose concentrations to "normal" ruminant levels.

Serum cholesterol levels in the control lambs decreased up to eight weeks of age and then stabilized. Commencing with the total removal of milk from the diet, the serum cholesterol levels of the gnotobiotic lambs increased dramatically until the end of the experiment. At 10 weeks of age, the serum cholesterol level in the gnotobiotics was nearly triple the level found in the controls. The high serum cholesterol values found in the gnotobiotics is in agreement with the observations of many other experimenters. However, the great divergence in cholesterol concentrations beginning at six weeks might be partially attributed to the decreasing feed intake of the gnotobiotic lambs.

While total lipid content of the livers of gnotobiotic and normal control lambs was practically identical, liver cholesterol was 68 percent greater in the gnotobiotics. The higher levels of serum and liver cholesterol in the gnotobiotics is best explained by the apparent accelerating effect of the intestinal microflora on the conversion of bile acids and cholesterol to insoluble forms which are excreted.

In further studies, lambs delivered by hysterectomy were reared in rigid plastic isolators. These lambs were fed a ewe's milk replacer, Ewelac, sterilized in its dry form with gamma radiation. Two control groups were removed from their dams at two days of age and placed in individual cages. One group (CSM) was fed the radiation sterilized Ewelac, while the other group (CEL) was fed nonsterile Ewelac. These groups of lambs were fed *ad libitum*. A third group of eight control lambs (CE) were left with their dams in the flock. Bi-weekly body weights and fasting and fed blood samples were obtained on all lambs throughout the eight week experimental period. The blood samples were analyzed for whole blood glucose, hemoglobin and plasma cholesterol. Various lambs from the three Ewelac fed groups were tested for fasting and fed levels of plasma total protein, liver lipid and cholesterol content and rumen fluid VFA concentrations.

Control lambs fed nonsterile Ewelac had the largest stomachs as a percentage of body weight and the largest rumino-reticulums as a percentage of total stomach weight. Gnotobiotics had the least developed total stomach and rumino-reticulums. The gnotobiotic lambs typically lacked any appreciable papillary development and had very thin stomach walls.

Gnotobiotic lambs had the greatest total gain in body weight and highest feed consumption, while lambs in the CEL treatment group had the lowest weight gain and feed consumption of the milk fed lambs.

CE lambs had an average fasting blood glucose level of 63.1 mg/100 ml which was significantly higher ($P < .05$) than the level of 46.8 mg/100 ml found in the CSM lambs. CEL and G lambs had average fasting blood glucose levels of 54.8 and 56.3 mg/100 ml, respectively. The latter two levels were not significantly different from each other or any other treatment mean. Gnotobiotic and CEL lambs displayed a relatively constant fasting glucose level of approximately 55 mg/100 ml from two to eight weeks of age. When compared to the other Ewelac fed control groups, the gnotobiotic lambs had a higher average fasting blood glucose concentration.

Gnotobiotic lambs had an average fed blood glucose level of 181.8 mg/100 ml over the course of the experiment. This level was significantly higher ($P < .05$) than all other treatment means. Blood glucose concentration decreased with age in all treatment groups with the exception of a temporary increase of 11 mg/100 ml from two to four weeks of age in the CSM lambs. Gnotobiotic lambs, having relatively very few rumen micro-organisms and therefore little fermentation, exhibited the highest fed glucose level. The CE lambs exhibited the lowest fed blood glucose level at eight weeks of age. Any lactose ingested by the CE lambs was more likely to be fermented to VFA and therefore unavailable for absorption.

Treatments had no significant effect upon the mean fasting plasma cholesterol concentrations. There was a highly significant ($P < .005$) age effect. Cholesterol levels increased with age in all Ewelac fed treatment groups. CE lambs exhibited an increase in plasma cholesterol level from two to four weeks of age, but from four to eight weeks of age the plasma cholesterol concentration decreased from 104.3 to 80.5 mg/100 ml.

The average fed plasma cholesterol levels of lambs in all treatment groups were very similar to levels obtained in the fasting state. No significant treatment effect occurred, but there was a highly significant ($P > .005$) age effect. Fed cholesterol levels increased with age in all Ewelac fed groups, but decreased from four to eight weeks of age among lambs nursing their dams.

All Ewelac fed lambs had significantly higher ($P > .05$) fasting hemoglobin levels than CE lambs, but were not significantly different from each other.

Age and treatment X age effects were highly significant ($P > .005$). CE and CEL lambs maintained relatively constant fasting hemoglobin concentrations of approximately 10.2 and 12.2 g/100 ml, respectively. The hemoglobin level of CSM lambs steadily increased from 12.0 g/100 ml at two weeks of age to 13.3 g/100 ml at eight weeks of age. Gnotobiotic lambs exhibited an initial increase in hemoglobin from 12.0 to 13.8 g/100 ml between two and four weeks of age. The latter level was maintained for the remainder of the experiment.

Project No. C-43

EQUINE SERUM TRANSAMINASES AS RELATED TO MUSCULAR WORK

Fiber digestion in the horse or rabbit, as is the case in the ruminant, is dependent upon microbial fermentation and the cellulolytic activity of microbial enzymes. In the ruminant, the major site of microbial activity is in the forestomachs. This precedes the passage of ingestion to the abomasum and the small intestine; in the rabbit or equine, bacterial fermentation is most concentrated in the cecum and colon which are posterior to the stomach and small intestine. Experiments designed to study the activity of microbial populations in ponies and rabbits have been conducted.

Ponies fitted with cecal cannula were fed timothy hay or clover hay with or without oats as 25 percent of the diet. The grain feeding resulted in significantly ($P > .01$) higher total and viable concentrations of bacteria in cecal contents. Oat feeding reduced proportions of gas producing bacteria. Both forage differences and oats feeding influenced proportions of the various protozoal groups observed in cecal contents. Total volatile fatty acids (VFA) and acetate concentrations in the cecum were higher in clover hay fed than in timothy fed ponies. Oats feeding increased total VFA and propionic and valeric acid concentrations.

Ponies fed timothy or clover hay, with or without oats, for 30 days were sacrificed two hours after morning feeding. Ingesta samples were collected from the fundic and pyloric regions of the stomach and from the ileum, cecum and terminal colon. Bacterial fermentation had occurred in all areas of the digestive tract. Proteolytic activity per gram of contents was greatest in the ileum. The number of viable, total and cellulolytic bacteria per ml of ingesta were found in the cecum. Relatively few *E. coli* were present as a percentage of the viable and total numbers of bacteria in the various gut regions. The concentrations of VFA were greatest in the cecum (97.5 uM/ml) followed by the terminal colon (25.6) and lowest in the stomach (14.3). It is evident that bacterial populations are active in all areas of the equine digestive tract and that the bacterial and protozoal populations of the cecum are affected by diet.

Studies designed to determine the effects of exercise upon various physiological parameters in ponies have been initiated. Apparatus for mechanical exercising and for measuring respiratory exchange during exercise are being developed.

A surgical method for cecectomy in the rabbit was developed. Studies to determine the effects of removal of the cecum upon digestive processes and blood chemistry have been conducted. Cecectomy resulted in reduced feed consumption and significantly lower ($P < .05$) weight gains than were observed in control rabbits of the same age. In digestion trials, hard and soft feces were separately collected and analyzed for gross energy, dry matter and acid detergent fiber (ADF). Coefficients of digestibility for dry matter were lower in cecectomized rabbits, though there were no significant differences in fecal ADF levels, indicating that the percentage of cellulose digested was not appreciably altered by cecectomy. A rate of passage study using chromic oxide as an indicator showed no significant differences between cecectomized and control rabbits in rate of passage of ingesta. Blood samples were periodically taken and analyzed for serum glucose, serum cholesterol, SGOT, serum sodium, potassium and phosphorus and for total serum protein, albumin and globulin. There were no significant differences due to removal of the cecum in any of the ten components.

Project No. C-44

A STUDY OF THE USE OF MEASUREMENTS FOR THE EVALUATION OF BEEF BREEDING STOCK

A number of outstanding results have been obtained from this project.

The potentiality of using measurements taken on live beef steers to predict the weights of the wholesale cuts of their carcasses has been found to be great and more accurate than the only standard for comparison which could be found - that of using packers' percentages of the carcasses.

The animals used were two groups of 100 steers each: one group weighed between 900 and 950 pounds and the other between 1,000 and 1,050 pounds, when measured and then slaughtered. Details of the materials and methods used are given in Bulletin A-150. The packers' percentages are the average ratios of the weight of a wholesale cut to the weight of the carcass. For example, the round equals 23.75 percent of the carcass and the rib, 9.5 percent.

Fourteen bulletins have been published from this project, 12 of which are within the years covered by this report. Too much information is available to allow giving details of the results in all 12 bulletins in this present publication. In general, for single wholesale cuts for the 900 and 1,000 pound groups, usually 10 to 14 measurements were found to be necessary to predict the weights of the cuts. By using measurements of the live animals, much more accurate estimates were made of the actual weights of the wholesale cuts than were possible using the packers' percentages.

Three combinations of cuts were also predicted for each group of steers: (1) Round, loin and rib; (2) Round, loin, rib and arm chuck; and (3) Round and loin. The results relative to the combined cuts for the 1,000 pound steers will be used to illustrate the results obtained. Thirteen measurements were found to be useful in estimating the weights of the combined cuts and a coefficient of multiple correlation of 0.85 was obtained relative to the cuts and the measurements. The average weight of cut (1) was 150.95 pounds. The average difference between the actual and estimated weights of the cuts was only 2.6 pounds, with the greatest error of estimate being only 8.53 pounds. When using measurements, 65 percent of the weights were estimated within 3.0 pounds of accuracy. When using the packers' percent, only 51 percent were estimated within 3.0 pounds of actual weight. The largest error in estimating the actual weight of the combined cuts (1) was 14.7 pounds when using the packers' percents compared to the 8.53 pounds when using measurements. In comparing these accuracies of estimating the weights of the cuts, it should be remembered that the packers' percent was applied to carcass weights while the measurements were made on live animals before slaughter. The variations in fill, weight of hide, weight of offal, etc., were not removed when estimates were made from using measurements as they were in applying the packers' percent to the carcasses.

This approach of using measurements to predict the weights of wholesale cuts has been developed for the first time by workers at the University of Maryland. It is a technique which could be tried in actual practice primarily by breeders trying to make real improvements in germ plasm. As this use of measurements is a new idea, more work needs to be done on animals of varying weights, sizes, sexes, breeds, etc., and various methods of cutting the carcasses must also be considered.

Other important findings from this project include: The parts of the animals which people usually observe when making visual evaluations of probable cut-out values are frequently not the parts which the measurements show are the important ones. These studies indicate that it might be advantageous for judges of livestock to re-evaluate their concepts. Another finding is that animals must be measured by means of instruments as significant dimensional differences can not be detected by eye.

The use of measurements to describe animals and to communicate in an accurate manner their lengths, depths, widths, ratios, etc., needs no further research and the practice should be used by livestock people in order to get exactness in their communication with customers and others.

A study has been initiated and a manuscript is being prepared concerning the differences in dimensions between breeds within the weight groups and between weight groups within breeds.

Project No. C-45

“CARBOHYDRATE UTILIZATION”

Carbohydrates have traditionally supplied the major source of energy for livestock diets. The carbohydrates which supply the greatest useful energy for animal growth (the production of lean red meat) are starches. These carbohydrates can also be effectively utilized by human beings; thus, it is essential that the utilization of carbohydrates be thoroughly understood so that these energy sources can be effectively directed toward human or animal consumption.

The utilization of carbohydrates, both those that can be utilized by humans (starches) and those that cannot (cellulose and hemicellulose) have been studied in pigs. It has been shown that pigs can utilize cellulose and hemicellulose to a greater extent than previously thought. However, when these carbohydrates are major dietary components, pig growth is not competitive with that

from animals fed predominantly starches. A surgical technique has been developed which allows the installation of cannulas at each end of the small intestine of pigs. Ingesta can thus be sampled after passing the stomach, small intestine or large intestine. Using this technique, the site and extent of digestion of various carbohydrates has been explored. When diets were based on forages, up to 75 percent of the total dry matter was digested. Of this, 55-60 percent took place in the small intestine, the remainder in the large intestine. Nearly 100 percent of the cellulose digestion took place in the large intestine, 80 percent of the hemicellulose digestion took place here. Neither the site nor the extent of digestion was related to the dietary hemicellulose-to-cellulose ratio.

When diets were based on grains, up to 80 percent of the total dry matter was digested. Of this, 78-86 percent took place in the small intestine. All of the soluble carbohydrates (starches, amylose, amylopectin and sugar) were predominantly digested in the small intestine. In a study of the rate of digesta passage through the digestive tract, it was observed that material spent the least time in the small intestine where most of the digestion took place.

In order to further evaluate the contribution of carbohydrates or other nutrients through gastrointestinal absorption, it is necessary to measure the rate of blood flow from the intestine to the metabolic system. A technique for measuring blood flow through the portal vein and posterior vena cava of pigs has been developed. This involves surgical preparation of the animal, infusion of a marker dye, withdrawal of blood samples, analyzing these samples for dye content and computing flow rates from these data. Considerable effort has been expended in refining the laboratory analyses and in writing a program for computer reduction of the data. This technique shows considerable promise in allowing more complete evaluation of nutrients and diets containing them.

Project No. C-46

STUDIES OF PARASITE CONTROL IN THE EQUINE GASTROINTESTINAL TRACT

A pilot project was initiated in January of 1972 to study the population characteristics of helminthic endo-parasites of horses (*Parascaris equorum*, Family Strongylidae, et.al.) in central Maryland. During the spring and summer of that year horses were acquired for inclusion in the research, techniques of collection and processing of samples were refined and preliminary data were assembled and evaluated.

Information generated during the pilot study seemed to indicate a regular, rhythmic oscillation in the population of helminthic endo-parasites. These cyclic variations strongly suggested an optimum worming schedule, particularly in view of the apparently marked effect of route of delivery of anthelmintic. Because of ambiguities in data caused by an inability to correlate fecal egg counts with parasite loads, post mortem examinations were added to the project.

In September, 1972, the project moved into a more definitive phase with the addition of eight weanling foals, thus giving a more complete age spectrum from which to draw data. At this time, two groups were selected for comparison of effect of route of anthelmintic administration (use of stomach tube and administration in feed). The groups were so drawn as to be as similar to each other as possible. Concurrently, an effort was begun to determine an optimum worming schedule for horses kept under certain conditions common to this area. At the same time, data was gathered from several herds outside the university, in order to verify the non-uniqueness of the school herd. A regular program of post mortem examinations was instituted.

Data available up to the present suggest a marked effect of atmospheric conditions on the parasites under study. For this reason, and because of the increasingly apparent value of post mortem examinations, the study will be continued through the summer of 1974. In the interim, outside herds will continue to be monitored.

Project No. C-48

SELECTION FOR CARCASS MERIT – BEEF CATTLE

The beef animal has been considered a source of choice meats from biblical times. The per capita consumption of beef in the U.S. indicates that it is the preferred meat of the American consumer. The high esteem for beef as our number one meat came about because it has been consistently tasty, tender and nutritious.

With the concern over fat in the diet, the production of excess fat beyond that required for eating satisfaction, necessitates the need for producing quality lean beef. The continuing success of the beef cattle industry is due primarily in giving the consumer a constant supply of the meat they like. Therefore, it is necessary that everyone concerned with the production and breeding of beef cattle continue to maintain a quality product that can be produced efficiently.

Beef carcasses vary a great deal in the percentage of lean, fat and bone. Although the consumer is interested in only the lean portion; fat is necessary for the production of tasty meat. Fat contributes juiciness and flavor to beef, and has a protective effect in the distribution, storage and cooking.

Conformation, finish and dressing percent have been the principal factors affecting the price of slaughter steers of the same age. However, the value of a beef carcass depends upon two factors: the acceptability of the meat and the amount of salable meat the carcass will yield, particularly the yield of high value preferred retail cuts from the round, loin and rib. These two characteristics, quality and cutability, of the beef carcass are not perfectly related. Therefore, quantitative and qualitative measurements must be made in beef carcass evaluation. Our beef cattle improvement program will continue to emphasize quality and cutability. In order to make more progress toward these objectives, arrangements are being formulated with the Maryland Correctional Institution located at Hagerstown for processing our beef. This will enable us to get detailed carcass information which in turn will be used as a selection criteria in our improvement program. Improvement in the overall performance of the University of Maryland beef cattle herds is vital to our carcass objective, and to production efficiency of the beef cow. In order to improve the individual performance and the production efficiency of our cow herds, only high performance tested bulls have been used over the last few years. The use of A1 breeding has enabled us to obtain the services of two outstanding performance tested bulls with adjusted 205 day weights of 693 lbs. and 759 lbs.; 365 day weights of 1,113 lbs. and 1,312 lbs. These bulls are now being mated to daughters of performance tested bulls.

Project No. C-49

“OVULATION CONTROL IN CATTLE AND SWINE”

In order for the animal industry to continue providing the increasing human population with an economically competitive supply of quality protein in the form of lean red meat, more control must be exerted over the processes that determine efficiency of animal production. The processes that are presently least efficient are reproductive processes. Many efficiencies can be gained through exerting some control over the process of ovulation such as: allowing more accurate timing of insemination, allowing the effective use of existing artificial insemination technology, more effective planning of the number of offspring to be born and the time that they will be born, and more efficient use of management, labor, facilities and other resources.

One example of such control is to cause an entire group of females to exhibit estrus and to ovulate at nearly the same time (synchronized). This has been accomplished with a high degree of success with both cattle and swine. The compound that has worked very well to synchronize estrus in swine is I.C.I. 33828 or methallibure. This compound is no longer available due to the very low probability of its receiving FDA clearance. Thus, even though we have achieved excellent synchronization of estrus in swine with no evidence of impaired fertility, the present outlook is not so promising.

Additional studies with cattle have indicated that either oral administration of melengesterol acetate (MGA) or the subcutaneous implantation of a progestinimpregnated plastic cylinder will effectively synchronize estrus, but fertility was substantially reduced. The reasons for the reduced fertility are not clear. In an attempt to further understand this phenomenon, samples of blood plasma were assayed for progesterone. Low levels of plasma progesterone were observed 10-14 days after estrus suggesting that premature regression of the corpus luteum (ovarian structure which secretes progesterone) may have caused the lowered conception rates. Studies are presently underway to determine if, in fact, this was the case. If so, the reduced conception rate should be easily overcome and considerable improvement in reproductive efficiency realized.

Project No. C-50

HORSE HOOF CHARACTERISTICS, THEIR CONTROL AND MODIFICATION FOR FUNCTIONAL DURABILITY

Economic loss attributable to poor hoof health in horses is inestimable. Idle time, loss of riding and racing time, veterinary and blacksmith expenses, treatments and medications comprise tremendous losses. Weak and brittle hooves are generally considered to cause lameness and unsoundness, such as quarter cracks, seedy toe, contracted heels, corns and tender feet. Secondary lamenesses more serious than the primary may develop from excess stress or improper treatment.

Hoof management and treatments have evolved historically without either basic or applied controlled research. Reliance principally has been placed upon observations, recommendations and practices of farriers and veterinarians. Proper hoof moisture is considered to be a principal factor in hoof health and function. Many commercial products are sold to increase moisture. Neither what constitutes proper moisture level in a horse hoof nor the means for producing and maintaining it has been established. Logic would indicate that excessive moisture, as well as excessive dryness, would be detrimental to the hoof.

Horsemen for many years considered horses having white feet to have soft poor quality feet, while a darker colored foot was considered to be harder and more durable.

Five horses, each having a black and white foot, were used to compare the mean hardness of white and black hooves when the mean amount of moisture in the horn is varied. These horses, when compared as to the effect of color on the hardness of the hoof wall, showed a curve characterized by a rather small variation in mean hardness above 20 percent mean water content and a rapid rise in hardness below 19.5 percent mean water content.

The percent moisture in the hoof wall of live horses was found to range from 20.81 percent through 44.71 percent with an average of 29.73 percent. In order to compare the results statistically, only those values above 19.55 percent moisture were considered.

A black foot and a white foot taken from the same horse were compared in regard to the effect of varying moisture content and its effect on the hardness of the hoof wall. No difference was found ($P > .05$). A total of five horses were compared as a group relative to the effect of white and black horn on hardness. No difference was found ($P > .05$).

The effect of age on the hardness of the hoof wall was considered. Horses two years of age and under were compared with horses four years of age and older, relative to mean hardness and mean percent moisture in the hoof wall. When the percent moisture in the hoof wall ranged from 19.56 to 32.08, the feet of the horses two years of age and under were softer than those of the horses four years of age and older. This difference was significant ($P > .01$).

Project No. C-51

PROJECT REPORTS

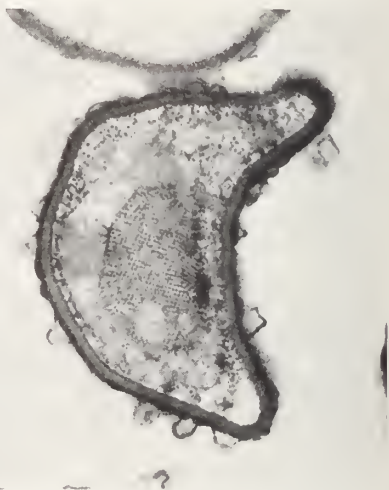
BOTANY

PLANT CELL WALL GLYCOPROTEINS AND CONTROL OF CELL ELONGATION

Primary cell walls of higher plants are rich in the enzyme peroxidase. Using a new assay, the distribution of cell wall peroxidase in soybean seedling hypocotyls was studied. Cell wall peroxidase is least active in the hook region, and most active in older hypocotyl tissue. Up to 25 percent of the peroxidase can be extracted from the cell wall by mixtures of wall-degrading enzymes secreted by fungi. The specific fungal enzyme that releases peroxidase has not been identified. The extracted peroxidase has a hydrogen peroxide concentration optimum different from that of cytoplasmic peroxidase. Also, the extracted peroxidase migrates in electrophoresis in multiple bands different from those obtained with peroxidase from cytoplasm.

Indirect evidence indicates that cell wall peroxidase is not the same protein as the hydroxyproline-containing protein of plant cell walls. First, incubation of cell walls with fungal enzymes releases up to 25 percent of the peroxidase of soybean hypocotyls, but only five percent of the hydroxyproline. The corresponding percentages for sunflower hypocotyls were 80 and 14 percent. Secondly, boron deficiency in sunflower seedlings resulted in an altered wall structure that allowed extraction of more hydroxyproline, but no more peroxidase, than controls. These differential extractions indicate that the two proteins are different from each other.

The ferrous ion chelator -dipyridyl increases the growth rate of soybean hypocotyl segments for a three-hour period. The length of this period can be increased by removing ethylene, which is made by the plant tissue and which stops cell elongation. Thus, the growth-promoting effect of the dipyridyl has been separated from the growth-inhibiting effect of the ethylene induced by the dipyridyl. The growth-promoting effect of dipyridyl is probably related to inhibition of hydroxylation of proline in cell wall protein. The normal hydroxylation is the first step of several reactions which result in a more rigid, and thus less expandable, cell wall.



Electron micrograph of an ultrathin section of a *Penicillium stoloniferum* spore showing virus-like particles within the cytoplasm of the spore. Magnification 31,500 X.

Project No. F-22

PLANT VIRUSES

During the past five years, viruses have been detected by electron microscopy in plants of *Rhoeo discolor*, *Haemanthus* species, *Jasmine* species, *Euonymus fortunei*, *Cattleya* and *Cymbidium* orchids, and in spores and mycelium of *Penicillium stoloniferum*. The virus particles of the Rhoeo disease are rigid rods approximately 300 mm long. The Rhoeo virus is serologically related to the type strain of tobacco mosaic virus, but has a different host range. The Rhoeo isolate does not infect tobacco plants. The virus-like particles associated with the Haemanthus and Jasmine diseases are flexuous rods.

The virus of *Euonymus* ringspot is icosahedral and is mechanically transmitted to a wide range of hosts. It does not infect tobacco plants systemically, but causes a severe systemic disease of petunias and cowpeas. The virus is transmitted by the nematode *Xiphinema* and is serologically related to tomato ringspot virus.

The most commonly occurring viruses detected in orchids are isolates of cymbidium mosaic virus (flexuous rod virus particles) or the orchid strain of tobacco mosaic virus (rigid rod virus particles). Electron microscopy of Cymbidium plants exhibiting a chlorotic mottle of the foliage did not reveal either the flexuous or rigid rod virus particles. A virus with an icosahedral particle has been isolated from diseased cymbidium. The virus is mechanically transmissible to a wide range of hosts. Its relationship to other viruses of plants is under investigation.

Electron microscopy of ultrathin sections of conidial spores and mycelium of *Penicillium stoloniferum* (ATCC No. 14586) showed virus-like particles packed in hexagonal crystals within the cytoplasm of the spores and mycelium. Similar virus-like crystals were not found in the spores or the mycelium of isolate No. 10111. Other fungi, especially those that produce pathotoxins or mycotoxins, are being examined by electron microscopy for the presence of viruses.

Figure 1 is an electron micrograph of an ultrathin section of a conidial spore of *Penicillium stoloniferum* showing virus-like particles.

Project No. J-98

FOREST TREE SEEDLINGS AND SOIL FUNGI RELATIONSHIPS

Various pine species are increasingly being used in the stabilization of completed sanitary land fills. The decomposition of organic materials in such areas results in the production of large quantities of gasses, some of which are known to be harmful to plant growth. In order for pine trees to grow successfully, their roots must be infected by mycorrhizal fungi. These fungi are very sensitive to the kinds of gasses produced from sanitary land fills.

The objective of this study was to determine the possible effect of low concentrations of methane gas on the growth of pine seedlings and on the establishment of mycorrhizal relationships. When air, as well as methane, was found to inhibit root development of pines, the determination of this additional inhibitory effect was incorporated into the study.

Various flow rates of air, and air plus one-percent methane gas, were passed through sterile, nutrient-saturated, Vermiculite-peat moss contained in flasks. After treatment of the medium for six days, 16-day-old Virginia pine seedlings were planted in these flasks. Some flasks were also inoculated with *Amanita rubescens*, a fungus commonly mycorrhizal with Virginia pine. Seedlings were maintained under continuous illumination for 30 days, and roots were examined to determine their degree of development and/or mycorrhizal associations.

Root systems of trees planted in flasks without *A. rubescens* were increasingly well developed with increasing air flow and reached maximal development at a flow rate of 0.5 liters per hour. At higher flow rates root development was almost totally inhibited. When one-percent methane gas was passed through similar flasks, root development was inhibited at all flow rates.

The experiments were repeated using flasks inoculated with *A. rubescens*. Root development increased with increased flow rate of air to a maximum of 0.75 liters per hour. The total root development was much greater than in similar experiments without *A. rubescens*. When one-percent methane gas was passed through flasks containing *A. rubescens*, roots of pine trees planted into the flasks developed at flow rates below 0.5 liters per hour, but increased sharply at higher flow rates.

Low concentrations of methane gas and to a lesser extent air without methane produce soil toxins inhibitory to root development in Virginia pine. The mycorrhizal fungus, *A. rubescens*, can partly counteract the effect of these gasses. Although some increased root development could be attributed to actual mycorrhizal infection, infection was not a prerequisite for detoxification of the medium.

Project No. J-101

DEVELOPMENT OF IMPROVED STRAINS OF MARYLAND TOBACCO RESISTANT TO DISEASE

The development of disease resistance in Maryland tobacco is a continuing process of crossing varieties with resistant parents. Following this, a process of selection for type, quality and constant indexing for resistance is made primarily by selfing.

Prior to 1969, the varieties released with resistance to the black shank disease were Md 59 and Md 609. Black shank is caused by a root rot fungus *Phytophthora parasitica* var. *nicotianae*. The two varieties were also shown to have resistance to fusarium wilt and black root rot caused by *Fusarium oxysporum* f *nicotianae* & *Thielaviopsis basicola*, respectively, both fungi. By 1970, over 55 percent of the tobacco growers were producing Md 59 and Md 609. Both varieties have low nicotine and nor-nicotine content and have excellent quality. Md Catterton, a grower selection, has resistance to fusarium wilt and a fair resistance to black root rot, it also has excellent quality.

A new variety released in 1969, Md 10, has resistance to tobacco mosaic virus, fusarium wilt, and black root rot but is susceptible to black shank. Md. 10 was developed from Catterton and a TMV (41-2) resistant parent, having Md Wilson characteristics, by tobacco breeders of the USDA in cooperation with the Agronomy Department. A Catterton x Wilson cross, Md 64, has also been released by the Agronomy Department with resistance to fusarium wilt. Md 64 is a broadleaf plant and produces a high yield. Md Wilson, a grower selection, is a high yielding tobacco with good quality but has no resistance to diseases particularly root rots. The above varieties probably make up over 90 percent of all the Type 32 Maryland tobacco grown in Maryland.

In recent years Md 59, Md 609, Catterton, Moore and Gertz, having been crossed with a tobacco mosaic virus resistant pollen parent 41-2 with Wilson characteristics, has resulted in many selections of TMV resistant plants. Through back crossing, reciprocal crosses and selfing the following crosses have been produced: (609 x 41-2) x 609, 609 x 41-2, 59 x 41-2, 59 x (41-2 x 59) x 59, (41-2 x Md Gertz) x Md Gertz, and Catterton x 41-L, (41-2 x Md Moore) x Md Moore with Maryland Type 32 characteristics, having 100 percent hypersensitive local lesion resistance to tobacco mosaic. All the selections from the above TMV resistant material in replicated field trials during 1970, 1971 and 1972 have shown 100 percent resistance to TMV. The selections with Md 59 and Md 609 parentage have shown 60-80 percent resistance to black shank. All have a high degree of resistance to fusarium wilt and a fair amount of resistance to black root rot. Some of the selections show a high per acre yield comparable to most commonly grown Maryland varieties with good quality.

Some of the selections have a real potential for Maryland characteristics acceptable to the tobacco industry as the breeding line M872. This line has a higher yielding Md 609 characteristic with quality equal to Md 609. It also has a good level of resistance to TMV, wildfire, fusarium wilt and a moderate level of resistance to black shank plus a better root system than the Md 609. Greenhouse indexing has indicated the levels of resistance acceptable to Maryland production. Md 872 should be a good asset to Maryland tobacco production.

One of the major functions of the varieties with disease resistance has been the lessening of disease losses due to fusarium wilt, which is difficult to find in fields, black root rot and black shank generally are found only occasionally in field plantings of susceptible varieties. Tobacco mosaic will, in a few years, be difficult to find. During 1973, many selections will be screened for TMV resistance, quality and yield in field tests. Since 1969, thousands of individual plants from many crosses and selections have been indexed for resistance to the major diseases found in Maryland tobacco.

Although there has been a major effort to produce resistance to a number of tobacco diseases, with a definite measure of success through the cooperation of the Botany and Agronomy Departments of the University of Maryland, and with the tobacco workers of the USDA, there are still problems to be solved, some old as greenspot (*Cercospora nicotianae*), and a new appearance of an old disease, tobacco etch virus (severe). During the summer and winter of 1972-73, all the Maryland varieties, and many selections, along with 56 species of *nicotiana* have at the present time shown no resistance in the Maryland varieties and selections. The species of *nicotiana* are being indexed at present writing. Literature has indicated that some species appear to be immune. Some Maryland selections do show differences in susceptibility.

The new varieties have been published in The American Journal of Agronomy, bulletins, and Fact Sheets.

Project No. J-103

EFFECT OF FUNGICIDES ON CELLULAR METABOLISM AND VEGETABLE DISEASES

Fundamental aspects of the toxic action and practical performance of several fungicides were studied. Benomyl, a prominent new systemic fungicide, breaks down to two products, both of which are fungitoxic. One of these products, Methyl-2-benzimidazole carbamate (MBC), is the compound primarily responsible for fungitoxic properties of benomyl preparations. The other product, butylisocyanate, is highly unstable, but may contribute to transient protective action of benomyl preparations. The toxic product MBC acts specifically on the mitotic process in a manner similar to colchicine. The 2-trifluoromethylbenzimidazoles act in a manner distinctly different from MBC. The former compounds uncouple oxidation from phosphorylation in fungi and other organisms.

Several compounds were found which selectively inhibit cyanide resistant respiration in *Ceratocystis ulmi* and *Ustilago maydis*. The most effective members of this group were 8-hydroxyquinoline and benzohydroxamic acid. Such compounds may be useful in controlling mutant pathogens which have a by-pass to metabolic blocks in the normal respiratory pathway.

Certain trisubstituted methanol derivatives, such as triarimol, are highly effective inhibitors of powdery mildew and other fungi. The site of action of triarimol has been localized to the pathway of ergosterol biosynthesis in fungi. Studies are now underway to determine the particular enzymatic reaction affected. *Cladosporium cucumerinum* mutants resistant to triarimol are also resistant to ancymidol, an analogue which is a plant growth regulator.

Chloroneb, a compound used to control Rhizoctonia root rot, affects membranes and wall structures in *Ustilago maydis*. A mutant of this fungus tolerant to Chloroneb is also tolerant to Botran and orthophenylphenol. Tolerance in this case appears to result from a mutation which confers resistance to structurally unrelated hydrocarbon compounds.

In practical tests chlorothalanil effectively controlled several important diseases of cucurbits and tomatoes occurring in Maryland. Benomyl has proven to be extremely effective in the control of powdery mildew, a serious disease of cucurbits, especially in cantaloupes. A three-year study has shown that full season disease control of tomatoes can be achieved with two timely applications of Difolatan, each at the rate of 2 gallons per acre. This method will lower the cost of a disease control program on tomatoes by reducing the number of spray applications required during the season.

Project No. J-91

THE APPEARANCE OF A HIGHLY VIRULENT FUNGUS DISEASE OF CORN DURING THE PERIOD 1969-1972

The appearance of a leaf blight in small areas of the country in 1969 did not disturb many people. However during the winter of 1969-1970, University of Illinois researchers determined that the disease was highly virulent, a form of the Southern corn leaf blight (SCLB) fungus *Helminthosporium maydis*. Up to this time, the fungus caused only sporadic damage because most hybrid corn had been moderately resistant to the disease. Two forms of the fungus were found. One form was highly parasitic on corn with the Texas male sterile (Tms) cytoplasm, but mildly parasitic on the normal (N) corn hybrids. The other form was mildly parasitic on both T and N corn. The virulent form was called "t" race and the mildly virulent form was called "o" race.

To become epidemic as this disease did in 1970 and 1971, several conditions are necessary. First, there must be a highly virulent parasite. Secondly there must be a susceptible host grown extensively over large areas. The Tms corn was present in about 85 per cent of the plantings in the United States. These two conditions plus favorable weather in the South during the early season of 1970 allowed a buildup of the fungus in the Southern corn areas. Because the fungus race "t" was a prolific spore producer, billions of these spores were ready to blow North to attack the larger corn producing areas.

In Maryland surveys indicated that the SCLB did not start until August of 1970. The heaviest infection was on the Eastern Shore and was progressively lighter as it travelled west into the higher elevations. The epidemic of 1970, which destroyed about 15 per cent of the corn, created the heaviest losses in the South and Midwest and indicated that the same could be expected in 1971. All the seed corn for 1971 would have a high per cent of the Tms cytoplasm. To avoid having the same situation occur in the South in 1971, most of the N resistant corn was sent South to be grown. This cut down on the heavy inoculum of the 1970 year.

Although the SCLB fungus is primarily a leaf disease, it also infects root, stalk, husk, cob and kernel. If the kernel is infected, the fungus may cause a seedling blight killing the young plant. Tests showed that of 574 seed lots brought into Maryland 27 per cent had infected grain. This means that many farmers bought fungus infected corn grain which could not be destroyed by seed treatments because the fungus was inside the grain. So far as could be determined, only the "t" race of the fungus would infect the grain. A number of isolations of the fungus were made from seed and leaf lesions on agar medium. The fungus race "t" produces a toxin which inhibits root growth of Tms hybrids, but not roots of N corn. Race "o" toxin will not inhibit root growth of either Tms corn or N corn. When infected kernels were placed on wet filter paper in petri plates, the fungus spores were produced overnight. Small hard structures were produced. These were later determined to be sclerotia and was the first time these structures were observed for the *H. maydis* fungus.

Surveys in 1971 indicated that there was less than five per cent damage to corn due to *H. maydis* race "t". Fewer spores from the South, weather not favorable to rapid sporulation or germination and the use of more N corn than in 1970 were some of the reasons for this. During 1972, nearly all

corn in Maryland and the rest of the country was of N hybrids. Thus the "t" race did not have a good host and did no damage. During the surveys of Maryland corn, three diseases not reported on corn in Maryland were found. They are: *Cercospora* leaf spot, yellow leaf blight and anthracnose leaf spot, all fungi. These diseases were found in many fields during 1972.

Project No. J-93

Species and Varieties of Medicago Show Increased Resistance to Sclerotinia Root and Crown Rot of Alfalfa

Root and crown rot of alfalfa has been a limiting factor in growth of alfalfa in Maryland. *Sclerotinia trifoliorum* and *S. sclerotiorum* are the main fungi that attack alfalfa in the field during wet, cool weather or under a snow cover throughout the winter. The organisms produce tough black sclerotial structures which survive for years in the soil. During the fall, spore bearing structures are produced by the sclerotia. The spores in turn fall on alfalfa plants, germinate and infect the crop plant. Over the past 10 years twenty-two species of *Medicago* and numerous varieties and selections have been indexed for resistance. All prove to be susceptible in varying degrees. Resistance that has been found has been in the root and crown. Foliage of all plants tested is highly susceptible. By 1969 all but a few species and varieties were eliminated, leaving the following species and varieties: *Medicago sativa* var. *gaetula*, *M. tianschanica*, *M. sativa* var. *Narragansett* and *M. sativa* var. *Williamsburg*. The first three of these have produced progeny that have increased in resistance to where certain selfed selections have, by 1972, up to 85 per cent survival. This is better than the Williamsburg variety which has been grown for many years as a resistant crop. Team, a variety that has resistance to anthracnose and weevil has no resistance to Sclerotinia. Although Team was included in the tests twice in three years, it has completely died out.

Three selections of Narragansett have been found with a high degree of resistance to red spider mite. There is a wide variation in resistance to this mite in a number of selections from above species and varieties. All the hardest selections from the 1971-1972 tests have been planted in plots at the Wye Institute on the Eastern Shore for observation on survival and seed production. Indexing will be run in 1973 on more selections.

A greenhouse and field study of four weed hosts of *Sclerotinia* sp. in alfalfa fields indicated that species of *Cruciferae* can be killed by this fungus in the seedling stage. The weed hosts, *Arabidopsis thaliana* had not been reported as a host before, while *Barbarea vulgaris*, *Lepidium virginicum* and *Capsella bursa-pastoris* had been reported as hosts. Older plants of the above genera and species of *Cruciferae* did not die from infection, but they acted as reservoir hosts. Twenty-four percent of *A. thaliana* collected from five alfalfa fields known to have a history of *Sclerotinia* were infected.

Project No. J-93

Soil Treatments with Chemical Fungicides May Increase Vegetable Crop Production

Control of bean root rot caused by the fungus *Rhizoctonia solani* appears to be a very difficult disease to control by the use of chemical soil treatments. Treatments with some 15 different chemicals at different rates and methods of application over the past four years indicate that this fungus is very difficult to control. Although certain chemicals allowed for better growth of the beans, most bean plants showed root rot at the end of the season. The population of the fungus was high for 1969-1972. In 1969 high rainfall appeared to retard progress of the disease. Much work is needed to find a chemical that will give adequate control.

There appears to be some promise of an effective control of sweet potato scurf causal organism, a fungus, *Manilochaetes infuscans*, by the use of chemical dips on the rooted sprouts. Over the last four years a number of chemicals gave very good to excellent control of this disease. Benlate alone and Benlate plus Folicate combination over the last two years, 1971-1972, have given excellent control.

The broad spectrum soil fumigants, Vorlex, DD-chloropicrin and Telone-chloropicrin, during the four year period 1969-1972, were evaluated as preplant row treatments in field plots for the control of the soilborne pathogen *Streptomyces ipomoeae*, the causal agent of sweet potato pox. Results indicated that all the chemicals reduced incidence of pox infection, however, not to acceptable low levels necessary for growers use.

A study of certain soilborne *Pythium* species associated with stem blight of bean *Phaseolus vulgaris* indicated that *Pythium ultimum* could be isolated from diseased beans during June, July and September, while *P. aphanidermatum* could be isolated during July and August. Both fungi could be found in the soil throughout the year. Disease development of the latter organism required high moisture in the field. Infection and development of the disease by spores or mycelium required the presence of water. It was found that none of nine species of *Phaseolus* inoculated with *P. aphanidermatum* were immune. Some lines of *Phaseolus lunatus* and *P. mungo* were more resistant than 138 cultivars and lines of *P. vulgaris*. Some cultivars and lines of *P. vulgaris* gave evidence of resistance to stem blight, while some lines which had been previously listed as resistant were found to be susceptible in this study.

Field tests have shown that a number of broad spectrum multi-purpose chemicals can be used for soil treatments to control nematodes, fungi and insects.

The experimental chemical AC64475, when applied to tobacco soil, infected with root-knot, stunt, stubby root, dagger and pin nematodes, as a granular material and disked in, gave good control over a 60 day period at four rates. The control was as good whether the treatments were made two weeks before planting or at planting time. The chemical is a systemic and will control aphids and flea beetle for most of the growing period. Some reduction of growth occurred in all treatments.

Root-knot nematode control on tomato roots conducted in field trials over the four year period 1969-1972 with contact and systemic compounds indicated that the compounds nemacur, temik and furadan generally increased tons per acre and gave good to excellent control of the root-knot nematode.

The nematocides nemacur, mocap and vorlex gave satisfactory control of root-knot on a susceptible sweet potato. They also increased yields, indicating that root-knot was controlled long enough during the growing season to permit good growth response.

Project No. J-93

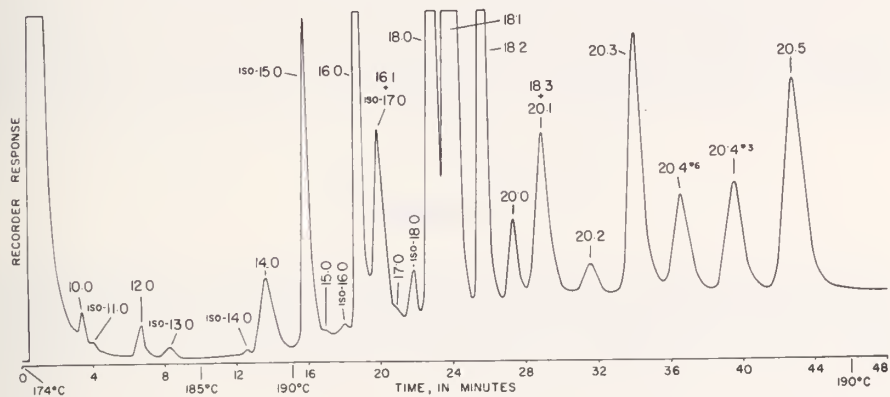
PHYSIOLOGY AND BIOCHEMISTRY OF NEMATODES AND NEMATODE-HOST RELATIONSHIPS

Nematode Lipids

Lipids, or fats, because of their importance as stored food for nematode survival, have continued to be studied. Active stages of most kinds of nematodes contain large amounts of lipids, ranging from 20 to over 50 percent of the dry weight of nematodes. Nematode eggs may be 65 percent lipid.

Comparative studies of lipids, primarily fatty acids, have been made of the free-living vinegar eelworm, *Turbatrix aceti*, and two species of plant parasitic root-knot nematodes, *Meloidogyne incognita* and *M. arenaria*. While mixed active stages of *T. aceti* contained around 24 percent lipid, root-knot nematode females contained 40-48 percent lipid, and eggs 64-67 percent lipid. Whereas *T. aceti* total lipid was composed of equal quantities of neutral and polar lipids, in root-knot nematodes it was over 90 percent neutral lipid. The major fatty acid fraction in both kinds of nematodes consists of 18-carbon monounsaturated fatty acids; in *T. aceti* this fraction is made up of equal quantities of oleic and vaccenic acids. In root-knot nematodes it is almost entirely vaccenic acid. It now appears that while *T. aceti* may be able to synthesize both oleic and vaccenic acids, plant parasitic nematodes may be able to synthesize only vaccenic acid. Figure 1 shows a typical gas chromatogram of the spectrum of fatty acids present in *T. aceti*.

Figure 1 — Gas Chromatogram of *Turbatrix aceti* Fatty Acids.



Lesion Nematode Control

The lesion nematode, *Pratylenchus penetrans*, is a destructive pathogen of a wide variety of plants in the Northeastern United States, including Maryland. Fumigant nematicides give poor or no control of this nematode in the heavier soils present in a large part of Maryland. Greenhouse tests in ground and raised beds containing heavy soil revealed that several non-fumigant nematicides provided excellent control of lesion nematodes on snapdragons and chrysanthemums. One of these pesticides, Temils R10G, has been released for use by commercial growers on several ornamentals; the optimum rate of application was 40 pounds per acre.

Project No. J-97

POSITION OF LIKE CHROMOSOMES BEFORE MITOSIS AND MEIOSIS IN CORN

Increase in the number of cells is accomplished by mitosis. The two cells resulting from the division of a single cell are identical in regard to the number of chromosomes, the physical carriers of inheritance. However, the chromosome number is exactly halved by a different type of division, meiosis, shortly before the formation of the sex cells. In contrast to mitosis, the two like chromosomes, derived from the male and female parents, pair or synapse in meiosis. Synapsis is essential for sexual fertility. It permits subsequent orderly distribution of the chromosomes to the sex cells. Little is known concerning differences in position of like chromosomes in cells before entrance into mitosis and meiosis, since chromosome structure is not evident prior to active division. However, at this time an identifiable cellular organelle, the nucleolus, is associated with chromosome six in corn. A single nucleolus is formed when the chromosome six from the male

parent and its counterpart from the female parent are relatively close together. Widespread separation of these two chromosomes results in dual nucleoli. Dual nucleoli were thirty-two times more frequent in cells entering mitosis than in cells entering meiosis. Accordingly, it is concluded that like chromosomes move near each other before the actual process of meiosis can be observed.

Project No. F-18

CROSSING—OVER IN CORN

Crossing-over, the exchange of hereditary factors between like chromosomes from the male and female parents, underlies the development of new genetic types in corn. The kernels of corn occur in paired rows, and side-by-side kernels have a common origin from a single protuberance on the young ear. It was found that the frequency of crossing-over is not related to the formation of kernels in pairs, but is a property of the entire ear. Three marker genes located in the second longest of the ten different chromosomes were selected for studies of recombination. Preliminary samples indicated different frequencies of single and double crossing-over for the testcross ears. Accordingly, the ears were divided into two equal groups with presumptive high and low frequencies and 3,534 seedlings classified. Single crossing-over was significantly higher, and an eight-fold increase in double crossing-over occurred in the high recombination group of ears.

PEPPER HAPLOIDS

Haploid plants, receiving inheritance from the female parent only, are useful for plant breeding and studies of plant genetics. The single set of chromosomes of a haploid can be doubled by the use of colchicine, an alkaloid, to restore the normal chromosome number of the species. Doubled haploids, produced in this fashion, are completely free from genetic variation, since both sets of chromosomes are identical.

Ordinarily, haploids are quite infrequent. However, one experimental line of pepper regularly produced approximately three percent haploid seedlings. This line has been increased to provide the experimental material for genetical studies. Hereditary factors controlling economic characters are believed to be often present in duplicate in a single set of chromosomes. Such duplications are difficult to detect by ordinary methods. However, the presence of duplications in the chromosomes of pepper can be demonstrated by studies of the haploids.

Project No. F-25

COMPARATIVE VEGETATIVE ANATOMY OF FLOWERING PLANTS

The most important plants to human economy are those which produce seed, the spermatophytes, which include the flowering plants. These are the plants with which man is primarily concerned in his desires to feed, clothe, and shelter himself and to improve and protect the quality of his environment. The microscopic structure of these plants (that is, the anatomy) is one of the keys to understanding the behavior of these plants and their interrelationships with each other and with the environment. Plant disease, properties of timber, mycorrhizal relationships, identification of wood, as well as the strictly esoteric disciplines of plant evolution and botanical relationships, are to a certain extent dependent on a knowledge of the internal structure of the plant. A continuing program to understand the structure of plants is of significance from the point of view both of the applied sciences and the basic sciences.

A major thrust in the Department of Botany has been oriented toward an understanding of the anatomy of the woody Saxifragaceae, a group of plants containing an alternate host of a serious disease of timber trees (white pine blister rust), many ornamentals (e. g., *Philadelphus*, *Hydrangea*,

Ribes, *Deutzia*, *Escallonia*), a few timber trees (e. g., *Polyosma* spp.), medicinal plants (e. g., *Dichroa febrifuga*, *Hydrangea arborescens*), and other plants which are strictly of scientific interest. A genus by genus investigation continues to be undertaken with publication following completion of studies in each genus. A summary of findings is outlined below.

Ribes

Leaves of *Ribes* are characterized by a trimerous vascular structure: the node is trilacunar, three traces enter the petiole, three veins supply the lamina at its base, and three veins are associated with each hydathode. Hydathodes are invariably present in the leaves of all species as are unicellular, bulbous-based epidermal hairs, and druses in the mesophyll. The xylem is characterized by scalariform perforation plates in vessels, transitional to opposite intervacular pitting, absence of axial xylem parenchyma, imperforate tracheary elements with circular bordered pits, high heterocellular vascular rays, broad and narrow rays in each species, and by the presence of isolated radially uniseriate groups of vascular ray cells throughout the genus. Bona fide axial xylem parenchyma has been noted only in *R. americanum* and simple perforation plates as well as scalariform perforation plates occur in *R. americanum* and *R. aureum*. The genus cannot be validly separated on anatomical grounds into two taxa, *Ribes*, *sensu stricto*, and *Grossularia*.

Escallonia

Leaves of *Escallonia* are all pinnately veined, unlobed, and dorsiventral except in *E. angustifolia* where they are isobilateral. Stomata occur only on the lower surfaces of leaves, except in *E. angustifolia* where they are present on both surfaces. Nodes are unilacunar and a single vascular trace enters the petiole and remains undivided throughout. Epidermis and palisade mesophyll vary from uniseriate to triseriate in different species. Druses are normally present only in the spongy mesophyll, except for a few species where they occur in both mesophyll layers. Hydathodes are found in all species except *E. herrerae* and *E. pendula* where their occurrence is doubtful. Unicellular, bulbous-based hairs occur on both leaf surfaces in most species; multicellular stalked hairs are also present on one or both epidermides of many species.

Xylem is characterized by scalariform perforations in the vessel elements of all species; in *E. millegrana* and *E. micrantha*, simple perforation plates are present as well. In these species only, there is ring-porosity. Spiral thickenings occur in vessel elements and/or tracheids of all species except *E. millegrana*, *E. micrantha*, and *E. pulverulenta*. Intervacular pitting is predominantly transitional; scalariform, opposite, and some alternate pitting are also present. Pores are mainly solitary. Vascular rays are uniseriate/homocellular and biseriate-multiseriate/heterocellular. All species possess apotracheal parenchyma; in *E. millegrana* and *E. micrantha* paratracheal parenchyma is also a regular feature of the xylem. On anatomical grounds, five species groups can be distinguished: 1. *E. pulverulenta*, *E. millegrana*, *E. micrantha*, *E. polifolia*, *E. myrtilloides*; 2. *E. angustifolia*; 3. *E. chlorophylla*; 4. *E. discolor*, *E. resinosa*; and 5. *E. herrerae*, *E. pendula*.

Studies on the anatomy of other spermatophytes have been carried out under the general program of comparative anatomy of flowering plants.

Cassytha

Studies on the angiosperm parasite *Cassytha* (Lauraceae) have shown that phloem is probably present in the haustoria of this plant and that the parasite derives food and water from hosts. In addition, it has been demonstrated that there are many points of similarity between *Cassytha* and

other members of Lauraceae. The unusual type of secondary growth which occurs in the stems of *Cassytha* in conjunction with the formation of haustoria is related to the amount of xylem present in the host plants. Haustoria were found to consist of two distinct parts, a "mantle" and a "penetration wedge," each of which arises endogenously from separate meristematic areas of the parasite stem. There are direct vessel to vessel contacts between the xylem of the host and that of the parasite. Anatomically, the genus *Cassytha* is very homogeneous.

Espeletia

Members of the genus *Espeletia* (Compositae) are anatomically homogeneous. The woods of all species studied possess distinct pores; vessel elements commonly have simple perforations, but occasionally there are multiple perforations. There are thin-walled, living libriform wood fibers, scanty paratracheal axial xylem parenchyma, and nearly exclusively multiseriate xylem rays in which erect and isodiametric ray cells generally predominate. Intermediate fiber/ray cells frequently ensheath multiseriate rays. Leaf structure is modified in response to varying degrees of physiological dryness, characteristic of the habitat in the high country of northwestern South America. Such xeromorphic modifications as increasing indumentum, development of hypodermal tissue, convolution of the abaxial blade surface, and production of either sclerophyllous mechanical support tissue or succulent water storage tissue, are characteristic of the leaves in most species. There is no anatomical basis for the separation of two growth forms (that is, arborescent and caulirosulan species).

Woods of Florida Keys

Studies of the anatomy of the secondary xylem of trees and shrubs from the Florida Keys have included Anacardiaceae, Burseraceae, Meliaceae, Rutaceae, Sapindaceae, Simaroubaceae, and Ulmaceae.

Ulmaceae

Anatomical investigations of the wood and leaves of Ulmaceae have shown that the tribes Ulmeae and Celtideae are more or less distinct, but the many transitional forms negate the formation of separate families based upon these taxa. Ulmaceae appears to occupy a primitive position in the order Urticales. Wood anatomy sustains the placement of Urticales near Hamamelidales and Juglandales rather than near Malvales and Rhamnales. Because of several specialized features in the xylem, Ulmaceae is considered to be moderately advanced to advanced phylogenetically. Wood anatomy supports the separation of *Chaetoptelea* from *Ulmus*. Ring-porosity and spiral wall thickenings in vessels are considered structural modifications correlated with seasonal environmental conditions in Ulmaceae. Scalariform perforations are reported for the first time in the vessels of *Celtis*, *Hemiptelea*, *Zelkova*, *Planera*, and *Ulmus*. The utilization of characters from the spongy mesophyll, cystoliths, crystals, mineral placks, and mucilage-containing cells in the leaf appear to be useful taxonomically.

Drosera

Drosera (Droseraceae), an insectivorous plant, bears stalked glands or "tentacles" which secrete and subsequently bear a clear, viscid droplet of mucilage at the apex to which insects adhere and become entangled. The tentacles are of two morphological types: radially symmetrical "disc tentacles," which are usually erect on the adaxial leaf surface, and bilaterally symmetrical

"marginal tentacles" borne along the periphery of the leaf margin. Tissues of the tentacles were found to be homologous with those of the leaf blade, that is, epidermis, mesophyll, and vascular tissues were represented. The marginal and disc tentacles, although differing in position, symmetry, and general form, produce glands which are homologous. The early development of both marginal and disc tentacles is identical until the glandular head becomes apparent. Each tentacle arises as a small, peg-like meristem which develops upon the adaxial surface of the leaf primordium. Differentiation of the tentacle meristem ultimately reaches the point where the glands are produced distally by the meristem; the stalk differentiates from those portions more proximal. The stalk comprises an epidermis originating from a protoderm, a mesophyll derived from the ground meristem, and a single vessel arising from procambial tissue. The glandular head of marginal and disc tentacles is comprised of four, distinct tissue layers. The epidermal layer differentiates from the protoderm. Subtending the epidermal layer is a layer of small, parenchymatous cells derived from the ground meristem that originated from the leaf primordium. Beneath this layer is a third layer which is distinctive in that it comprises elongated cells derived from the protoderm, and non-elongated parenchyma cells developed from the ground meristem. The fourth layer, which is a complex of tracheids, is derived from the procambium.

THE EFFECT OF NUTRIENT AND ENVIRONMENTAL FACTORS ON THE FORMATION OF LIPIDS IN PLANTS

The essential elements for most groups of plants, the levels of most of these elements which give good growth, and their roles are well known in plants. However, as our attention has been focused on the water pollution problem, we fail to fully understand the responses of unicellular algae to limiting concentrations of various nutrients or the biochemical effects of limiting concentrations of essential nutrients. This project has been conducted to answer some of these problems.

The nutrition of algae has been studied for a number of years, but it has not taken the form considered to be appropriate for higher plants—a concentration series experiment followed by a factorial experiment. The concentration series experiment is used to determine the levels for the factorial experiment. Others studying the nutrition of algae have simply described levels of various nutrients which gave an optimal growth rate, or have adopted the published medium and, in most cases, investigations into the nutrition of the alga in question ceased at that point.

The object of this work was to identify the quantitative requirements for Ca^{2+} , Mg^{2+} , K^+ , and Na^+ in the alga, *Chlorella sorokiniana* and to determine what biochemical irregularities occurred when each of these elements was present at a deficiency level in the plant, an approach not used before.

In the Mg^{2+} concentration series, Mg^{2+} was varied from 0.01 to 1000 meq/l. The lowest concentration was decidedly inhibitory to growth. The growth rate first reached its optimum at about 0.08 meq/l (sufficiency concentration) and toxicity was observed at concentrations above 100 meq/l.

The same type of response was seen in the K^+ concentration series experiment. The sufficiency concentration for K^+ was 0.1 meq/l and toxicity was observed at concentrations above 100 meq/l.

The requirement for Ca^{2+} in this organism has been demonstrated previously, but the level of Ca^{2+} required is extremely low. Although deficiency of Ca^{2+} could not be demonstrated in the present work, Ca^{2+} toxicity was shown at concentrations above 10 meq/l (Figure 1).

Using these data as a guide, levels of Mg^{2+} and K^+ were selected for a factorial experiment. Inasmuch as Na^+ was the variable cation in the series experiment, it was also included in the factorial experiment. The levels chosen for "High", "Medium", and "Low" were: Mg^{2+} ; 0.008, 0.03, 0.10 meq/l; K^+ ; 0.01, 0.04, 1.0 meq/l; Na^+ ; 0.01, 0.10, 1.0 meq/l.

Sodium had no effect on the growth rate or any of the biochemical factors studied. An optimal growth rate was obtained only when Mg^{2+} and K^+ were both "High".

Analyses of plant material, produced under various nutrient media, showed (Table I) that optimal growth rates were obtained only when the optimal cellular levels of K^+ and Mg^{2+} were present. In fact, levels of cellular Mg^{2+} and K^+ increased as the nutrient medium level increased until the sufficiency level was reached. Beyond this level an increased concentration in the medium had no effect on cellular levels.

TABLE 1
Effect of Cation Levels on Growth and Elemental Composition of *Chlorella sorokiniana*

Cation Level*			Growth Rate (Doublings Per Day)	Mg^{++} as % Dry Wt.	Mg^{++} as Meq/100 g Dry Wt.	K^+ as % Dry Wt.	K^+ as Meq/100 g Dry Wt.	Na^+ as % Dry Wt.	Na^+ as Meq/100 g Dry Wt.	Ca^{++} as % Dry Wt.	Ca^{++} as Meq/100 g Dry Wt.
Mg^{++}	K^+	Na^+									
H	H	H	9.0	0.20	16.4	1.2	30.7	0.02	0.90	0.36	18.0
H	H	H	9.0	0.23	18.9	0.99	25.3	0.02	0.90	0.34	17.0
H	H	L	9.0	0.23	18.9	1.1	28.1	0.01	0.44	0.33	16.5
H	M	H	8.3	0.15	12.2	0.60	15.3	0.03	1.3	0.50	25.0
H	M	M	8.3	0.13	10.7	0.58	14.8	0.02	0.90	0.59	29.5
H	M	L	8.2	0.14	11.5	0.55	14.1	0.03	1.3	0.53	26.5
H	L	H	6.9	0.13	10.7	0.29	7.4	0.05	2.2	0.46	23.0
H	L	M	6.8	0.15	12.2	0.27	6.9	0.03	1.3	0.42	21.0
H	L	L	6.7	0.15	12.2	0.29	7.4	0.01	0.44	0.45	22.5
M	H	H	7.8	0.18	14.8	1.0	25.6	0.05	2.2	0.26	13.0
M	H	M	7.7	0.16	13.1	0.86	21.9	0.05	2.2	0.33	16.5
M	H	L	7.8	0.17	13.9	1.2	30.7	0.04	1.7	0.33	16.5
M	M	H	8.2	0.16	13.1	0.59	15.1	0.03	1.3	0.50	25.0
M	M	M	8.1	0.15	12.2	0.49	12.4	0.02	0.90	0.55	27.5
M	M	L	8.0	0.15	12.2	0.52	13.3	0.02	0.90	0.54	27.0
M	L	H	6.5	0.15	12.2	0.33	8.4	0.03	1.3	0.49	24.5
M	L	M	6.4	0.15	12.2	0.34	8.7	0.03	1.3	0.67	33.5
M	L	L	6.4	0.14	11.5	0.25	6.4	0.03	1.3	0.78	39.0
L	H	H	6.7	0.11	9.0	0.49	12.5	0.03	1.3	0.37	18.5
L	H	M	6.7	0.10	8.2	0.38	9.7	0.03	1.3	0.44	22.0
L	H	L	6.9	0.08	6.6	0.32	8.2	0.02	0.90	0.24	12.0
L	M	H	6.8	0.10	8.2	0.46	11.8	0.03	1.3	0.46	23.0
L	M	M	6.8	0.10	8.2	0.43	11.0	0.02	0.90	0.37	18.5
L	M	L	6.8	0.10	8.2	0.38	9.7	0.03	1.3	0.36	18.0
L	L	H	6.6	0.08	6.6	0.17	4.4	0.02	0.90	0.26	13.0
L	L	M	6.5	0.07	5.7	0.15	3.8	0.02	0.90	0.29	14.5
L	L	L	6.3	0.08	6.6	0.12	3.1	0.02	0.90	0.28	14.0

* Magnesium = (L)ow, (M)edium, and (H)igh = 0.008, 0.03, and 0.10 meq/L, respectively
Potassium = (L)ow, (M)edium, and (H)igh = 0.01, 0.04, and 1.0 meq/L, respectively
Sodium = (L)ow, (M)edium, and (H)igh = 0.01, 0.10, and 1.0 meq/L, respectively

Mg²⁺ and K⁺ were both necessary for the synthesis of protein (Table II). When either element was lowered below the sufficiency level, a decrease in total nitrogen was observed in the cells.

Lipid levels were increased by K⁺ deficiency and decreased by Mg²⁺ deficiency. It is suspected that when K⁺ deficiency inhibited protein synthesis, extra carbon compounds were funneled into lipid synthesis. K⁺ deficiency was especially effective in inhibiting the synthesis of unsaturated fatty acids.

The nutrient medium sufficiency levels of K⁺ and Mg²⁺ were determined to be 0.1 and 0.08 meq/l, respectively. Deficiency levels of Na⁺ or Ca²⁺ could not be demonstrated. Potassium deficiency resulted in increased lipid concentration, although unsaturated fatty acid synthesis was inhibited along with protein synthesis. Magnesium deficiency resulted in inhibition of protein synthesis and lipid synthesis. No significant biochemical effects were observed with Na⁺ and Ca²⁺.

Project No. K-10

TABLE 2

Effect of Cation Levels on Growth and Biochemical Composition of *Chlorella sorokiniana*

meq Mg ⁺⁺ /L	Growth Rate (Doublings Per Day)	Total Nitrogen % Dry Wt.	Total Lipid % Dry Wt.	Total Fatty Acids % Dry Wt.	Saturated Fatty Acids % Dry Wt.	Unsaturated Fatty Acids % Dry Wt.	Unsaturated Fatty Acids/ Saturated Fatty Acids
0.01	4.7	4.9	22.5	4.3	2.8	1.2	0.41
0.05	8.5	7.1	18.7	3.8	1.7	2.1	1.2
0.10	8.7	8.9	22.5	7.9	2.2	5.7	2.6
1.0	8.6	9.0	19.4	6.4	1.8	4.4	2.5
10.0	8.6	8.9	19.2	5.6	1.6	4.0	2.5
100.0	8.6	8.4	16.9	4.3	1.5	2.7	1.7
200.0	6.9	7.2	14.8	3.1	1.3	1.6	1.4
500.0	4.6*	-	-	-	-	-	-
Meq K ⁺ /L	Growth Rate (Doublings Per Day)	Total Nitrogen % Dry Wt.	Total Lipid % Dry Wt.	Total Fatty Acids % Dry Wt.	Saturated Fatty Acids % Dry Wt.	Unsaturated Fatty Acids % Dry Wt.	Unsaturated Fatty Acids/ Saturated Fatty Acids
0.003	4.8	6.9	26.3	6.1	4.8	2.8	0.58
0.016	6.4	7.3	19.9	6.5	2.8	4.0	1.4
0.033	7.4	8.4	19.7	7.3	2.8	4.3	1.7
0.166	9.1	9.8	20.9	6.9	2.1	3.9	1.9
0.333	9.2	9.7	20.2	7.9	2.3	4.7	2.0
9.3	8.9	9.8	19.9	7.8	1.8	4.7	2.6
99.3	8.9	9.6	17.0	5.6	1.7	3.8	2.2
499.3	1.5*	-	-	-	-	-	-
meq Ca ⁺⁺ /L	Growth Rate (Doublings Per Day)	Total Nitrogen % Dry Wt.	Total Lipid % Dry Wt.	Total Fatty Acids % Dry Wt.	Saturated Fatty Acids % Dry Wt.	Unsaturated Fatty Acids % Dry Wt.	Unsaturated Fatty Acids/ Saturated Fatty Acids
0.001	9.2	9.2	22.9	9.6	2.8	6.6	2.3
0.101	9.2	9.8	20.8	9.1	2.9	6.1	2.1
1.00	9.2	9.7	20.2	9.2	2.8	6.3	2.3
10.0	9.2	9.0	19.8	7.9	2.4	5.6	2.3
100	8.3	5.6	13.1	3.8	1.9	2.5	1.3

* One replicate only. All other samples in duplicate

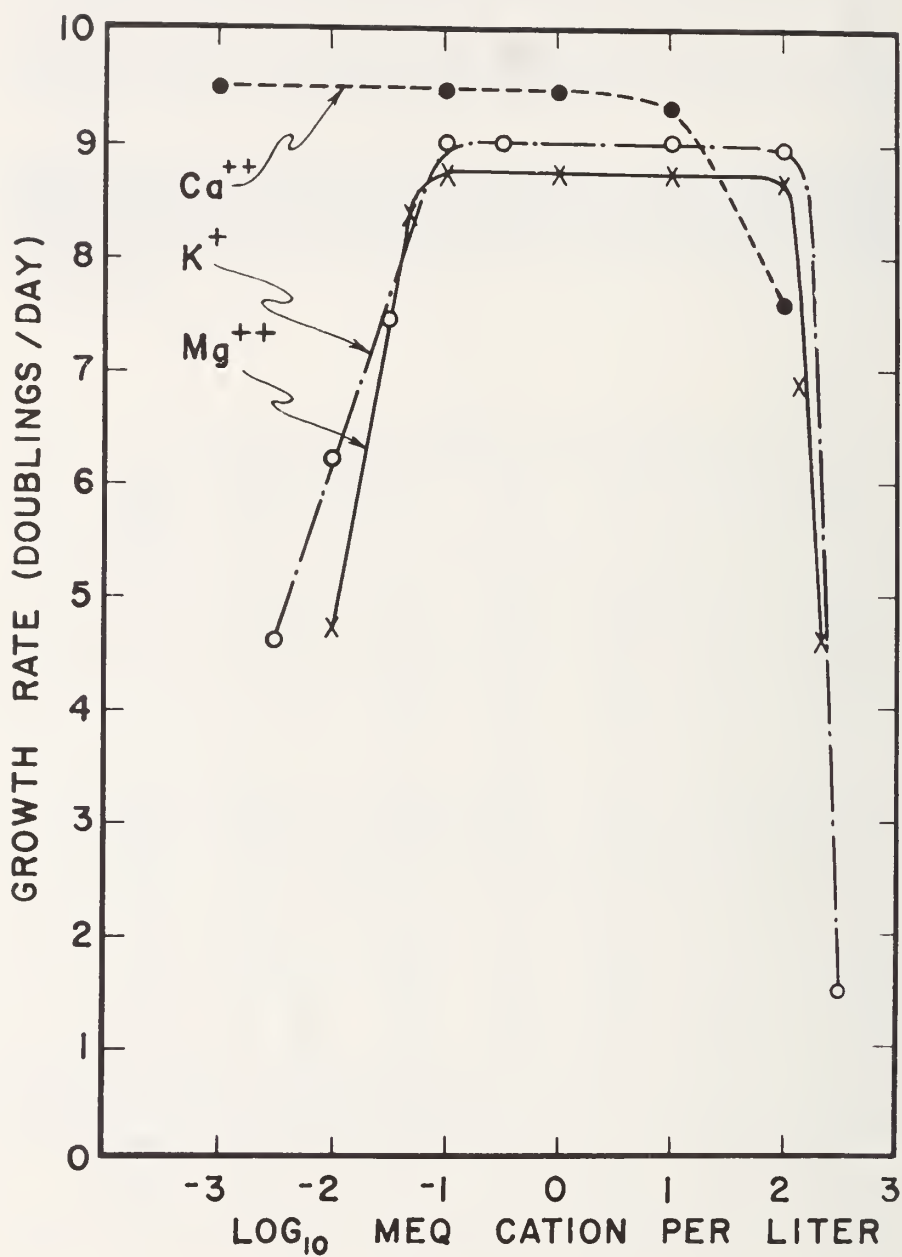


PHOTO BIOLOGY OF FUNGAL REPRODUCTION

The mechanism by which certain plant pathogenic fungi absorb radiant energy and translate it into a biological response is still a fundamental mystery. The nature of the photoreceptor is unknown. One of the methods that can be used to deduce the molecular nature of the photoreceptor in biological systems is to determine which wavelengths of light are most effective in stimulating the response (action spectrum). Once this is determined certain biologically important pigments become candidates for the photoreceptor because they absorb the wavelengths that are most effective in stimulating the response.

The nature of the photo response in *Nectria haematococca* was investigated in this laboratory. Techniques for obtaining the action spectrum for sexual reproduction were developed for this fungus. The fungus responds to exposure to radiant energy by forming sexual fruiting bodies called perithecia. The number of perithecia that form depended upon the length of exposure and intensity of irradiation.

A number of destructive plant pathogenic fungi have a similar photo-induced sexual stage. The formation of perithecia results in the liberation of airborne spores which are disseminated in nature and thus help perpetuate the spread of plant disease. Since natural sunlight contains the effective wavelengths, a study of the effects of radiant energy on sporulation may lead to a better understanding of how the organism may be controlled. Also, detailed action spectra of the sexual response in fungi are not common. The general shape of the action spectrum obtained for this organism was found to be similar to the action spectra obtained for a multitude of different plants. This probably indicates that a common photosystem exists in these organisms.

Project No. J-102

BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF OZONE ON PLANTS

Air pollution damage to vegetation has become an important widespread modern problem. This research is investigating the possibility that air pollution damage to crop plants may affect the normal resistance of plants to naturally occurring plant pathogens. In addition, biochemical changes in ozone-treated plants are being studied by high resolution electrophoretic techniques to determine if enzyme changes may be correlated with an altered plant disease resistance.

Project No. K-12

BIOSYSTEMATIC STUDIES OF NORTH AMERICAN VASCULAR PLANTS

Systematics is the study of the diversity of living organisms found on the earth, and is generally a comparative approach. Its role in biology is basic to the science. All other fields depend directly on systematics, and systematics -- in turn -- derives additional data from these fields whereby the systems of relationships of all plant and animal life can be more accurately determined. The purpose of the current study is to present basic information on the vascular plants of North America to the field of botany, and especially those members of flowering plants found in the western United States and northern Mexico.

Monographic Studies

Monographic studies are those efforts of systematics devoted to a single group of organisms, usually a genus or small group of genera. Attempts are made to learn as much as possible about the individuals. Currently, efforts associated with this program are being concentrated on the plant

genus *Eriogonum*, a member of the knotweed family Polygonaceae. This large genus is found mainly in the western half of North America and has some 240 known species. Although work on this group has been carried on since 1961, concentrated studies on the genus have been made in the field and in herbaria since 1970 resulting in six major publications during this period. Field studies have been conducted in north-central Mexico, Baja California and most of the western states of the United States. Herbarium visits have been made to major eastern North America collections and several in western Europe. A final, monographic paper will be published by 1976.

Preliminary efforts are currently being initiated on the genus *Salvia*, a member of the mint family Labiatae. Research will be conducted jointly with R.M. Harley of the Royal Botanic Garden, Kew, England. Our hopes are to monograph this large genus throughout the New World.

Floristic Studies

Intermountain Flora

The Intermountain Flora project is an attempt to determine the names and distribution of all vascular plants found in a large area of the western United States found between the Sierra Nevada on the west and the Rocky Mountains on the east. The first volume of this work was published in 1972, with the remaining five volumes to appear over the next ten to twelve year period. Each known species is described and discussed in detail with an individual drawing accompanying it. Field work during the last three years has concentrated on the aquatic monocotyledonous plants, and the treatment of these organisms has been completed. The summer of 1973 will be largely devoted to a review of the species associated with the lily family, Liliaceae.

Southwest Flora

The National Science Foundation has awarded a grant to prepare an illustrated, multi-volumed flora of the vascular plants found in the southwestern part of the United States. This area includes the desert regions of southeastern California, southern Nevada, most of Arizona, all of New Mexico, and a small portion of western Texas. Field work on this project is just getting started, and we hope to have this flora completed within fifteen years.

Flora of Maryland

Efforts are now being made to produce a student-oriented flora of the herbaceous plants of the state of Maryland. This project is being guided at the University of Maryland by R. G. Brown. Special efforts will be made to review the taxonomy of all treatments submitted, review original publications and see type specimens of as many of the species as possible, both in the United States and in Europe.

As a result of floristic studies, eight papers have been published describing new species in *Frasera*, *Lathyrus*, *Penstemon*, *Grindelia* and *Phacelia*, all from the state of Nevada. Important taxonomic notes have been published, and two new variants have been reviewed from Mexico. Additional papers on plants found in Mexico will appear in the near future.

Cytological Studies

Efforts have been made to determine chromosome numbers for various vascular plants found as part of the above floristic studies in the western United States. A small number of papers have been published to date enumerating this information. Additional reports will be made from time to time.

SUMMARY

Systematic studies supported by this project have concentrated on monographic studies of the genus *Eriogonum* and floristic studies of the Intermountain and Southwestern portions of the western United States, as well as Maryland. As a part of these studies, a program of reviewing and determining chromosome numbers of various vascular plants has been conducted. Future floristic studies will continue to emphasize the Intermountain and Southwestern floras, while monographic studies will shift to the genus *Salvia*. Cytological studies will continue as a part of the overall floristic program.

Project No. F-24

EFFECTS OF SODIUM CHLORIDE ON *CHLORELLA SOROKINIANA*

Summary

Incubation of this high-temperature strain of *Chlorella* in NaCl resulted in bleaching and destruction of lipids at 1.0 M NaCl while decreased growth, increased dry weight per cell, increased intracellular concentrations of Na^+ and Cl^- , more total chlorophyll per cell, a decreased chlorophyll A to chlorophyll B ratio, increased rates of oxygen evolution and decreased rates of carbon fixation were evident at 0.3 M NaCl. Requirements for light-energy were higher in NaCl inhibited cultures. Inclusion of Ca^{++} in inorganic medium extended the organism's tolerance to higher concentrations of NaCl. Calcium and light showed an interaction on the growth and intracellular ion content of the alga. This work indicates that application of calcium to saline-crop systems may stimulate yields.

Destruction of Unsaturated Lipids

Cells of this unicellular green alga were bleached after a 24 hour incubation in medium containing 1.0 M NaCl and irradiation with 10 Klux of white light from fluorescent lamps. All pigments and fats had been reduced to zero or trace amounts with the exception of a 16 carbon fatty acid. In medium of low salinity, this alga contains chlorophyll and carotenoid pigments and three major fatty acids. Two of the fatty acids are 16 carbons long and one is 18 carbons long. The 18 carbon and one of the 16 carbon fatty acids are unsaturated and designated as 16:2 and 18:2. Both of these fatty acids are reduced by the 24 hour salt incubation leaving the 16 carbon saturated fatty acid designated 16:0 as the only significant fatty acid.

Photosynthesis

Oxygen evolution in cultures of this alga was measured in inorganic medium with and without NaCl. The rate of oxygen evolution per cell, per chlorophyll molecule, and per dry weight was greater in the light-dependent region for cultures in 0.3 M NaCl. The light-dependent region started at a lower light intensity for cultures in 0.3 M NaCl. The apparent quantum yield for oxygen production in this *Chlorella* was increased by culturing in 0.3 M NaCl.

Growth, Dry Weight, Chlorophyll

The maximal growth rate occurred at 2.7 and continued up to 14.0 mW/cm^2 of white light. Inorganic medium was non-saline with 0.05 meq Ca^{++}/l . Saline medium was inorganic medium with NaCl incorporated. Inhibition of growth occurred in saline medium but was counteracted by increasing light intensity. White light of 9.4 mW/cm^2 was required to saturate growth in 0.3 M NaCl-saline medium. Saline medium compared to inorganic medium increased dry weight per cell

up to 2.2 times, reduced chlorophyll per dry weight, but increased chlorophyll per cell. A 400-fold increase in Ca^{++} concentration by incorporation of Ca^{++} salts to the level of sea water, 20 meq/l, produced Ca-saline medium. In this medium, growth occurred up to 0.5 M NaCl at 1.2 and 2.7 mW/cm² of white light. Without the high level of Ca^{++} in saline medium, complete inhibition of growth occurred with NaCl at 0.35 M and greater.

Intracellular Ions

Analyses of intracellular-ion concentrations showed Na^+ and Cl^- increasing and K^+ decreasing with increases in extracellular NaCl. Use of Ca-saline medium decreased intracellular Na^+ and Cl^- and increased K^+ . Similar results were obtained in saline medium by increasing light from 1.2 to 2.7 mW/cm². Ca-saline medium reduced intracellular Na^+ more than a light increase did, while intracellular Cl^- was reduced more by the increase in light than by increase in Ca^{++} . These intracellular-ion changes were greatest when levels of both Ca^{++} and light were increased.

Project No. K-11

BIOPHYSICAL AND BIOCHEMICAL FACTORS IN PLANT NUTRITION

"Nutritional Studies with Soybeans"

The nutritional requirements of soybeans have been studied extensively in hydroponic culture in a greenhouse. With this equipment the desired concentrations of various nutrients could be attained and maintained throughout the growth of the plants.

With Dare soybeans, a factorial experiment was conducted with three levels of nitrogen, phosphorus, and potassium. When plants in the seedling stage were inoculated with nodule bacteria, the plants receiving the highest concentration on nutrient-nitrate (620 ppm) formed few if any nodules; those receiving a medium concentration of nitrate (124 ppm) formed some nodules; and, those plants receiving the lowest nutrient concentration of nitrate (25 ppm) formed abundant nodules. However, the highest yields were obtained with plants receiving the highest nutrient concentration of nitrate even though these plants had no nodules. Regardless of the nutrient concentrations of nitrate and potassium, the lowest yields were consistently associated with the lowest nutrient concentration of phosphate (1.0 ppm H_2PO_4^-).

In an evaluation of interactions in these factorial experiments, it was demonstrated that yields could not be improved by increasing the nutrient-phosphate unless nutrient-nitrate was adequate. Similarly, yields could not be increased by additional nutrient-nitrate unless nutrient-phosphate was above a given level (10 ppm H_2PO_4^-) and hence adequate.

A good correlation was obtained between the nutrient concentration of potassium and the yield of soybean plants. There was no relationship between yield and the concentrations of potassium, calcium, or magnesium in the leaves. Per cent oil in soybean seeds was related to the nutrient supply of potassium. Per cent oil increased as nutrient-potassium was increased from 10 to 40 ppm. Variations in calcium and magnesium had no effect on the per cent oil in the seed.

Six soybean cultivars—Illington, Boone, Patoka, P1-65342, Kim, and Dorman—were studied with regard to their responses to potassium fertilization. Evidence by other workers had indicated that some cultivars were "potassium responders", whereas, others were "non-responders". In our studies, Dorman did not "respond" to variations in potassium nutrition. For the other cultivars, increases in potassium caused increases in: (a) yield; (b) growth of axillary branches; (c) number of

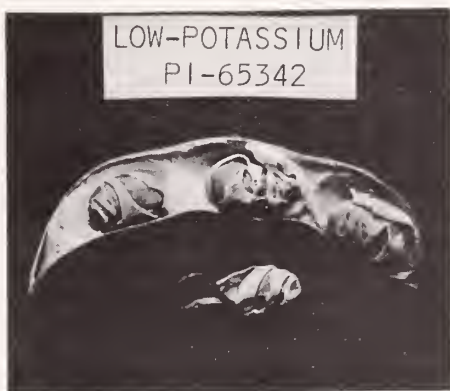
nodules; (d) seed size for Kim and Illington; (c) per cent oil in seed of four of the cultivars; (f) number of seeds per pod; and, (g) in the case of Corman, the number of 4-seeded pods. Incomplete seed coats appeared in certain varieties. With the lowest nutrient level (4 ppm) of potassium, the percentages of seeds with "incomplete" seed coats (see picture) were: Illington, 85; Patoka, 35; P1-65342, 60; and, Dorman, 99 percent. Increases in nutrient potassium supply essentially overcame this disorder in all cultivars.

For Cutler soybeans, receiving 0.025, 0.5, or 5 ppm boron (B) and manganese (Mn), in all combinations, the highest yields were obtained with medium levels of B and Mn in the nutrient solution. The treatments had no effect on the per cent oil in the seed. The concentration of Mn was twice as high in leaves of plants receiving 0.5 ppm B as in those receiving only 0.025 ppm boron. On the other hand, changes in Mn supply had no effect on the concentration of B in the leaves.

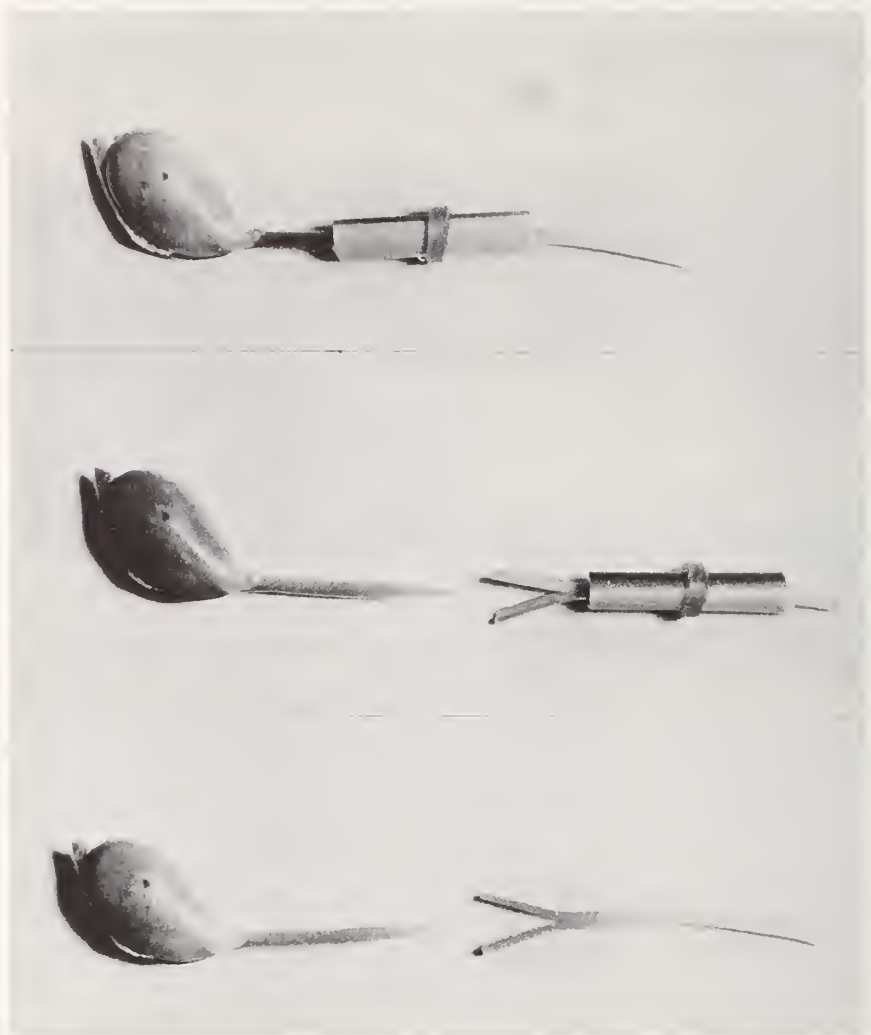
Grafts (see picture) were made between two types of soybeans so that the scion of a "chloride-includer" (S-100) type was grafted onto the roots of a "chloride-excluder" (CNS) type, and vice versa. A scion of each type was also grafted onto a root system of the same type in order to measure effects caused by grafting alone.

By using such grafted plants, it was possible to determine that the roots, rather than the scion, determined the concentration of chloride in soybean tops. However, the type of scion did have a lesser, but secondary, modifying effect on leaf-chloride.

Project No. K-8-C



With a low level of K^+ in the nutrient solution, the seed coats of the P1-65342 soybean cultivar, and certain other cultivars, were incomplete.



Technique for grafting soybean seedlings.

K-8-c: Biophysical and biochemical factors in plant nutrition.

PROJECT REPORTS

DAIRY SCIENCE

THE CHEMICAL MUTATION AND IMPROVEMENT OF DAIRY STARTER CULTURES

The importance of microbial fermentations to the manufacturing of dairy products such as cheese, butter, milk and yoghurt is well documented. The rapidity at which dairy starter cultures can form lactic acid is critical from both an economical and public health viewpoint. The shorter the fermentation time, the less cost and labor required and equally important, the more remote the possibility of contamination by pathogens.

The isolation and selection of improved cultures is currently being examined. It is possible to form mutants of currently available starters for the purposes of obtaining organisms which would exhibit a greater efficiency or a capacity not currently expressed by the parent organism. The chemical mutagens diethylsulfate, ethylmethane, N-methylhydroxylamine, and N-methyl-N-nitro-N-nitrosoquamide (NTG) have been used as potential mutagenic agents for isolating improved mutants. NTG was selected for further work on the basis of ease of handling and consistency of the data obtained. Two single strain cultures, *Streptococcus lactis* JL, and *Leuconostoc citrovorum* RA, were isolated from the commercial buttermilk culture and exposed to NTG. Conditions of exposure which permit a predictable surviving percentage of the two cultures have been established. The probability of obtaining a desired mutant was improved under these conditions. Mutants with increased glycolytic and proteolytic activity have been isolated.

Project No. G-58

CONTROL OF REPRODUCTION IN THE BOVINE FEMALE

The likelihood that prostaglandins would be available for use in controlling the reproductive cycle generated research on them. Preliminary studies have been conducted in postpartum cows with the prostaglandin F_2 to determine the effective systemic dose. Material was only available to permit the treatment of four cows. Based on the occurrence of estrus and changes in blood progesterone concentration, doses of 20 micrograms per kilogram body weight do not appear effective, while doses above 25 micrograms per kilogram body weight appear to be effective. Estrus occurs 70 to 78 hours post treatment. A 50 percent conception rate was obtained on first breeding. Use of the prostaglandins for control of reproduction will require data on tissue and milk residues. Preliminary data have been obtained from lactating dairy goats using tritium labeled prostaglandin. These preliminary data suggest that residues may be present in milk for at least 48 hours post treatment.

A major problem in dairy cattle is unsuccessful early rebreeding of the postpartum cow. Because the prostaglandins may prove ineffective or too expensive for use in cattle, other techniques are being studied to give breeding "by appointment". Forty-five cows have been given various treatments using estradiol, progesterone, PMS and HCG with breeding about day 40 postpartum. Several of these treatments gave conception rates high enough to warrant further study.

Because of reproductive abnormalities noted in a number of the cows in the studies above, it was decided not to pursue the treatment schemes further until a considerable amount of "normal" baseline data on blood progesterone concentration postpartum had been obtained. Twice weekly blood samples for more than 60 cows have been obtained for the first 60-70 days postpartum, including both normal cows and those showing evidence of endometritis. Samples analyzed to date indicate that blood progesterone concentration did not exceed two nanograms per ml until an

average of 39 days postpartum. This increase was not preceded by estrus. Estrus did not appear to occur unless the plasma progesterone concentration was elevated above two nanograms per ml for ten days to two weeks.

This finding is suggestive of the first cycle of the breeding season in the ewe. Because progesterone may be useful as part of a controlled breeding "by appointment" sequence in cattle, further attention has been given to the fecal excretion of progesterone metabolites in the form of androgens. The potency of androgens in chick assay systems has been known for many years. We have now shown that the androgens in incubated cow feces are orally effective in laboratory mice. The effects include blocking of estrus cycles in non-pregnant females and abortion in pregnant females. These findings posed two problems: (1) Would these androgens be present and active in proposed composting systems for barn wastes? (2) What are the ecological implications for wild rodents? Preliminary data with composted barn waste showed no evidence of this androgenic activity. The androgens may be destroyed by the heating and oxidation of the composting process. This is being pursued at greater sensitivity to determine whether composting does away with this potential problem. Studies to examine for androgenic effects on several wild rodents are underway.

The use of progestin ear implants for the control of reproductive cycles has been examined in two trials in 32 beef cows and heifers. A synthetic progestin, SC-21009, from G. D. Searle Company, was implanted at a dose of six milligrams for 14 days. Estrus synchronization appeared good. Conception rate appeared to be an approximately normal 56 percent. However, these conceptions appeared to be the result of natural service to follow-up bulls. Because of apparent breakthroughs to estrus on the replication, another trial will be conducted using a 12 milligram implant rather than the six milligram implant used in these studies.

Project No. G-57

FACTORS AFFECTING ENERGY AND PROTEIN INTAKE AND UTILIZATION IN RUMINANTS

A series of model studies has been conducted using laboratory animals to determine the effects of fat (as a caloric input) and the sex hormones (estrogen and testosterone, endogenous and exogenous) on energy intake and utilization. Methods used were the comparative slaughter technique and open circuit calorimetry (indirect). The results indicate that males and females respond differently to dietary fat. Growing females regulate intake at a lower level than males. Subsequent studies with intact and gonadectomized males and females showed that intake reduction was closely related to estrogen levels (fed, as diethylstilbesterol and removed, by ovariectomy). The hormone influence is under investigation in dairy cows to determine whether residual estrogen load early postpartum can explain poor appetite during this period. Interactions between estrogen and calcium, sodium and potassium are under investigation as they relate to central nervous system control of intake in cattle and in rats using experimentally induced hypothalamic and diabetes.

Energetic efficiency in male rats was greater than in females and cannot be explained by the composition of tissue gained. This interesting and unanswered phenomenon is under study.

An open circuit respiration chamber for small ruminants has been completed and will be used in studies to determine the effects of meal patterns, intake and concentration of various nutrients and metabolic end products on energy metabolism. Unique features of the chamber include flexibility in design to accomodate a size range from laboratory animals to animals weighing 600 to 800 pounds.

Project No. G-56

ANALYSES OF PRODUCTION AND FEED DATA FROM DAIRY RECORDS

A total of 3,285 yearly (1959-1967) herd average records from Maryland DHI Holstein herds were examined to determine the relationship between various herd variables and milk production or income over feed cost. Variables included in the study were herd size, body weight, feeding rates for concentrates and forages, milk and feed prices and percent days in milk.

The amount of concentrates fed, percent days in milk, body weight and rate of roughage feeding were found to have the greatest influence on milk production. Of these variables, the amount of concentrates fed was 2.5 times as important as the other three. For each 100 pound increase in the amount of concentrates fed per cow annually, milk production increased by 82.4 pounds. A 100 pound increase in body weight resulted in 468 pounds more milk produced. A one percent increase in days in milk increased milk production by 109 pounds.

Analyses of data for relationships with income over feed cost included the following five variables: milk production, milk price, forage cost, amount of concentrates fed and grain price. Milk production was found to be the most important variable. If milk production is given a value of one, the relative importance of milk price was 0.6, of forage cost was 0.4, of amount of concentrate fed was 0.4 and of grain price was 0.2.

Project No. G-54

UTILIZATION OF COTTAGE CHEESE (ACID) WHEY

Studies were initiated to incorporate fluid acid whey, a nutritious by-product of cottage cheese production, in existing and new food and beverage items. Because of the acid character of this whey, items with natural acidic properties provided the greatest potential. Products studied were: whey wine, acid fruit flavored drinks, cocktail mix bases, jellies and jams and salad dressing.

A whey wine similar in flavor to Chianti was prepared from an alcoholic fermentation of acid whey. Whey was supplemented with various levels of lactose and sucrose to provide sufficient fermentable substrate to produce 12 to 13 percent alcohol. Using lactose supplementation without sucrose, a maximum of 10 percent alcohol was obtained. Through usage of commercial lactose enzyme preparations and lactose fermenting yeasts, the desired alcohol content should be obtained with the lactose substrate.

Fruit flavored drinks and cocktail mix bases were successfully formulated. Drinks containing equal volumes of fluid whey and water properly sweetened, acidified and flavored received higher proportions of whey. Whiskey sour and daiquiri cocktail mixes that duplicated existing commercial products were prepared from fluid acid whey flavored with lemon and lime concentrate extracts properly balanced to the desired sweet-sour characteristics with sucrose and citric acid. Work is continuing on drinks and mixes to prepare them in a powdered form.

Jellies and jams were processed from sweetened, flavored acid whey using the short-boil pectin method. Flavors used were lemon, orange, lime, grape and fruit punch. Jams were prepared by increasing the level of whey solids in the acid whey prior to processing. Gelatin, flavor and storage properties of these products were excellent. Characteristic whey flavor was not detectable until whey solids were in excess of 10 percent.

Salad dressing, normally prepared with a water and oil blend, was developed by replacing the water with fluid acid whey. This blend possessed flavor characteristic of a seasoned vinegar-oil dressing. With this basic blend, dressings such as roquefort, French, Italian, Russian, thousand island, etc. can be prepared by the addition of appropriate flavoring components and condiments.

Project No. G-53

FLAVOR IN "MODIFIED" MILK

The level of polyunsaturated fat in milk is low in relation to some other sources of food fats. When highly unsaturated fats are fed to the dairy cow in a so-called "protected" form, the level of polyunsaturated fat in milk increases quite markedly. Such milks are very susceptible to developing oxidized flavor. Cooperative work with the USDA at Beltsville was undertaken to investigate the effect of vitamin E as a possible stabilizing factor in these special milks. Initial work involved supplementing the ration of the cows producing polyunsaturated milk (PUM). The results showed an anticipated increase in vitamin E in the milk; however, it only partially improved the milk stability. Additional research was concerned with the direct addition of vitamin E in an emulsified form. These results indicated the PUM could be stabilized, providing that vitamin E is added shortly after the milk is removed from the cow. This research also established that the severe fat deterioration in PUM did not occur until after the milk was removed from the cow.

Project No. G-48

OXIDIZED FLAVOR IN MILK

The role of vitamin E in relation to the stability of milk against fat oxidation and the resulting oxidized flavor was revealed in previous work on this project. The role of vitamin A or carotene in reactions involving fat deterioration was investigated during this period. Dairy cows were given daily supplements of beta-carotene along with their ration of alfalfa hay and grain concentrate. Analyses of the milks showed marked increases in carotene; however, the level of vitamin E was not influenced nor was the oxidative stability of the milk changed. These results indicate that vitamin A, unlike vitamin E, is not related to the development of oxidized flavor in milk.

Vitamin E is rather inefficiently used by the dairy cow with up to 50 percent of the intake excreted in feces and less than 2 percent being incorporated into milk. A study was carried out to determine if the amount of vitamin E absorbed from the digestive system could be increased by pre-treatment of the vitamin supplement. Considerable success was achieved when this approach was used to incorporate vitamin E by direct addition to milk. Tocopheryl acetate was pre-emulsified with Tween-20, based on preliminary studies using a model system. The daily supplement was given to four Holstein cows in a changeover design which included balance studies. Results indicated that the emulsification treatment did not influence the excretion of Vitamin E into feces nor its transfer to milk.

Project No. G-34

THE ANALYSIS OF DAIRY PRODUCTS

Detection Of Adulterated Ice Cream

A method for detecting adulteration of ice cream with vegetable fat was developed. It was based on the vitamin E method developed earlier in this project, but simplified for application in the usual ice cream plant laboratory. Products with obvious abnormal levels of vitamin E are presumed to contain fat other than milk fat.

In conjunction with the research on iron-fortified milk, methods were developed for the determination of iron and total vitamin A (vitamin A plus carotene) in milk.

A major effort was devoted to research and evaluation of a new automated instrumental system for measuring the fat content of milk - the Technicon Auto Analyzer. The system automatically and continuously samples, analyzes and records the fat content of 40 milk samples/hour. The project leader accepted the assignment as Associate Referee by the Association of Official Analytical Chemists (AOAC). Two collaborative studies involving six laboratories were conducted. The results were presented to the AOAC and as a result the Auto Analyzer system for milk fat determination has been granted First Action as an Official Method. The system can be used to analyze milk as a basis of buying and selling.

Project No. G-35

COMPONENTS OF COMMERCIAL COTTAGE CHEESE (ACID) WHEYS

Difficulties were frequently encountered in duplicating physical and organoleptic characteristics of product in whey utilization studies when the source of fluid whey was changed. In some instances, differences were noted on a day to day basis in whey from the same source.

Samples of whey from local commercial cottage cheese producers used in the study were microbiologically and chemically analyzed. Data showed that microbiological quality of acid whey, regardless of source, was excellent if cooking temperature was 50 deg. C or higher. No significant increase in microbial population was noted after 30 days storage at 20 or 5 deg. C.

Differences were noted in chemical composition of wheys. Components had the following ranges: butterfat - traces to 0.1 percent, protein - 0.65 to 1.3 percent, lactose - 4.3 to 4.9 percent, ash - 0.65 to 1.3 percent and lactic acid - 0.4 to 0.6 percent. Differences in soluble and non-soluble proteins were noted. Levels of denatured protein were high in the wheys cooked at temperatures higher than 50 deg. C. Rapidly cooled whey had lower levels of protein denaturation than those cooled statically. Product turbidity resulted from protein denaturation. Flavor defects were observed in all products made from whey with high ash and acid contents. These high levels were present in all wheys produced from skim milk supplemented with non-fat-dry-milk solids. Such supplementation is common practice to increase cottage cheese yields. Whey with ash content approaching one percent and/or lactic acid concentration above 0.5 percent required product formulation changes to eliminate flavor defects. Skim milk, to which starter distillate was added to increase flavor in cottage cheese, produced a strong flavor in whey that was difficult to mask.

The addition of acid whey to products with delicately balanced flavors without knowledge of the cottage cheese processing and whey handling methods poses serious product development problems. With knowledge of method of production used, if uniform on a day to day basis, appropriate formulation and handling modifications can be made to eliminate the possible defects due to differences in whey composition and characteristics.

Project No. G-35

A METHOD FOR DETERMINING PROTEIN CONTENT OF MILK

A study was undertaken to evaluate a new automated system for measuring the protein content of milk. The system, also a Technicon instrument, was compared to two accepted methods. Results suggested that several modifications are needed before a collaborative study and application for official status are in order.

In cooperation with a local food distributor, an evaluation of several techniques and instruments for measuring the fat content of ground beef was carried out. Results indicated that slight modifications of the Babcock Test for fat in milk could readily provide a method with application in the retail phase of meat marketing. A limited survey of market products indicated an urgent need for such a test.

Project No. G-35

PROCESSING FACTORS AFFECTING QUALITY OF ICE CREAM

Investigations were continued in the area of ingredient usage and their effects on ice cream quality. These studies involved corn syrup solids, cereal solids, whey solids as fresh liquid whey, condensed, dried and modified whey solids, sweetener solids, protein products, non-caloric ingredients and various special ingredients. The results have culminated in the development of numerous formulations to fulfill the dietary needs of consumers having various dietary restrictions.

The formulations have served a valuable function both commercially and in home made products. Formulations utilizing whey have provided a means to transfer significant amounts of this by-product from a contaminant in the environment to a useful food.

Project No. G-42

NUTRITIVE EVALUATION OF FORAGES

During the period covered by this report, research on several topics has been initiated and completed. Each topic is reported separately.

Forage Grasses

The effects of cutting data, variety (alfalfa) and fertilization (nitrogen) on yield, chemical components related to nutrient content, animal intake and digestibility have been measured on alfalfa and Midland Bermuda grass. Bermuda grass declines in nutritive value at twice the rate of alfalfa with age (0.5 vs. 1.0 percentage units per day). The alfalfa weevil resistant variety Team is equal to other varieties in chemical indices of nutrient content. Intake by animals is equal to that of other varieties evaluated. Of special value is the observation that leaves are held longer by Team than by other varieties.

Corn Silage

Evaluation of different varieties of corn silages (Pioneer 1097, Dekalb XL-45) harvested with and without ears, ensiled at different dry matter levels (20.5 percent to 49.4 percent), planted at high or low rates (21,250 to 43,900 plants per acre) and fed with or without soybean meal to steers and cows were conducted. Variety had little effect on intake, which was most related to dry matter content. The earless silages were consumed at lower rates and contained lower dry matter levels. Some difference between varieties in digestibility was observed. Feeding soybean meal did not result in a significant difference in digestibility of any nutrients. Planting rate did not have a consistent effect on intake or digestibility, but was not completely evaluated in these trials due to lack of adequate control groups.

Interest in the status of sulfur-amino acid adequacy for high producing cows prompted investigation of factors related to sulfur nutrition and total ration utilization and metabolism by animals. Preliminary *in vitro* studies showed a linear relationship between sulfur level and cellulose digestion up to 0.2-0.25 percent sulfur in the diet. Corn silage is low in sulfur. Thus,

supplementation is advisable to insure maximum utilization of cellulose and bacterial cell growth in the rumen. Balance studies with steers fed corn silage, corn and urea indicated that sulfate sulfur or methionine hydroxy analog improve ration digestion. When compared to an unsupplemented control, these supplements plus dl-methionine all improved nitrogen utilization. Dairy cows fed corn silage plus alfalfa hay showed no beneficial response to sulfur sources when studied after peak lactation. Complete blood amino acid profiles were gathered on all cows, but show no marked effects due to sulfur sources.

Caloric Density

Lactating cows were fed diets of differing proportions of concentrates and roughages to provide a broad range of digestible energy concentration (kcal/g) and density (kcal/ml). Intake measurements were made during the first half of lactation to compare the two methods of expressing energy content to predict voluntary intake as limited by gut capacity (intake vs. demand). Caloric density is superior to caloric concentration as a predictor for maximum intake. This finding is of great importance in formulating total rations for cows fed free-choice. Maximum utilization of forages and maximum energy intake are possible during early lactation.

Project No. G-47

ENDOCRINE FACTORS AFFECTING REPRODUCTION IN CATTLE

Study has continued to elucidate the mechanisms of early pregnancy that may fail and result in early embryonic mortality in cattle or which may be useful in providing techniques for the control of reproduction in cattle. Major emphasis has been placed upon the study and isolation of the luteolytic materials from cow uteri. The protein luteolytic material appears to have a molecular weight of about 70,000 based upon chromatographic evidence. Electrophoretic analysis indicates a minimum of five components in this preparation, three of which are identical with blood albumins. The luteolytic effects of these proteins may be brought about by the binding of small molecules. Data from other laboratories has suggested this might be steroids or prostaglandins. Radioactive label studies suggest only a low binding ratio with the steroid hormone estradiol. Cold extraction with acetone: methanol (five: one, v/v) at pH 2 did not destroy the luteolytic activity. Chemical analysis indicated only minor traces of prostaglandins in this preparation. Luteolytic activity has been found to be present in protein preparations from cow uteri during early pregnancy (prior to 45 days of pregnancy). The extraction data suggest that this activity is not due to prostaglandins being present. Data obtained from sheep in another laboratory have shown an increase in the luteolytic prostaglandins during early pregnancy. Data from cattle and sheep suggest that a mechanism must exist which prevents these luteolytic activities from functioning in early pregnancy. Failure of this mechanism may be a cause of early embryonic mortality. Conversely, the isolated luteolytic materials, protein or prostaglandins, may provide a reasonable means of controlling cattle reproductive cycles.

A cooperative study with the Agricultural Research Service has confirmed that extracts from 15 day old sheep embryos can maintain corpora lutea function when placed in the uterine lumen. This maintenance of corpora lutea function is required for the continuation of pregnancy in both the cow and the sheep. Death of the corpora lutea can be brought about by the presence of an intra-uterine device (IUD). The infusion of extracts of 15 day embryos into the uterus has been shown to prevent this corpora lutea death in the presence of an IUD. These data suggest that the embryo itself may act directly or indirectly to block the luteolytic effects of the uterus as described above.

Project No. G-50

IRON FORTIFICATION OF MILK

A major effort during this period was concerned with the development of an iron-fortified milk and its evaluation as a possible dietary supplement for iron. In the initial phase, numerous iron compounds were evaluated for their suitability. It was well known that both iron and copper added to milk can cause the development of oxidized flavor. Four compounds were finally selected for extensive evaluation in relation to their effect on the quality of the milk. The iron-supplemented products were subjected to both expert and consumer panels for flavor judgments and to several chemical tests in relation to possible deterioration of nutrient properties. Of the various iron compounds, only ferric ammonium citrate (FeAC) was found suitable. Additional studies were carried out using only FeAC. The milks were processed by laboratory and commercial systems. Results indicated the feasibility of producing a commercial product with up to 30 ppm of iron added to the raw milk just prior to processing as FeAC. These milks were as acceptable to the flavor panels (40 persons on the consumer panel) as the untreated milk and showed no losses of certain nutrient properties.

The final phase of this study was the determination of the biological availability of the iron in the iron-fortified milk to the consumer. Iron in some forms added to various foods is not absorbed by the animal. Baby pigs were selected as test animals, because their digestive systems are more similar to the human digestive system than some of the more common laboratory animals. The 12-day-old baby pigs were adjusted to a modified milk ration containing iron as FeAC and then given a dose of a ration containing radioactive FeAC. The use of radioactivity as a tracer allowed a rather complete evaluation of the fate of the iron in the iron-supplemented milk. The analyses indicated that 30 percent of the iron consumed in the ration was absorbed by the baby pigs. Over 90 percent of that was incorporated into red blood cells. Other studies of iron absorption from various foods indicated that only about 10 percent was utilized by the animal. These studies suggest that milk would be a suitable vehicle for introducing iron into the diet.

The levels shown to be feasible and the very favorable absorption indicate that about $\frac{1}{2}$ pint of iron-fortified-milk would provide the recommended daily intake for iron in the human diet.

Project No. G-48

PROJECT REPORTS

ENTOMOLOGY

THE BIOLOGY OF BITING MIDGES OF THE GENUS *CULICOIDES* (DIPTERA: CERATOPOGONIDAE) IN MARYLAND

Our studies during this period have concentrated on two genera of Ceratopogonidae (Diptera), *Culicoides* and *Forcipomyia*, with the most recent work being confined to *Forcipomyia*. Taxonomic review of the subgenus *Trichohoelea* was completed and an article published on it. Graduate students completed their studies and published two papers on *Culicoides* and one on the subgenera *Thyridomyia* and *Synthyridomyia*. Another student began field studies on the ecology of *Forcipomyia* which was halted by his being drafted into the army. Two undergraduate students completed a special project on *Forcipomyia* breeding sites.

A bibliography of Ceratopogonid feeding habits was begun and a paper on a new species of *Culicoides* from Colombia was prepared. A note on a range extension of *Aedes triseriatus* into Greenland was also published. An extensive review of the literature of Iceland and Greenland insects was assembled.

Project No. H-86

THE CONTROL OF BIRD DEPREDATION

The purpose of this project in Maryland was to find ways to reduce populations of depredating blackbirds, particularly the Red-winged Blackbird, and to develop techniques to reduce the damage inflicted on corn by depredating birds. Three major activities were undertaken to achieve our goals.

Chemosterilants can safely reduce numbers of birds, if administered in such a way that non-target species are not affected, and if the normal behavior patterns of the target species are not altered. Tests elsewhere using Ornitol (Searle SC-12937) on pigeons had been successful, so for three years we tested this chemical on a population of Red-winged Blackbirds on Kent Island (Queen Annes County) in Chesapeake Bay. In 1969, cracked corn, soaked in a solution of Ornitol, was placed on feeding platforms in a marsh from April until July. Nesting (hatching) success was observed in this marsh and a nearby control area. Only 18.1 percent of the eggs in the test area hatched, whereas 55.1 percent of the eggs in the control area hatched. Only one nest in the test area produced young birds. However, it was felt that a lower concentration could probably be used without a loss in effectiveness.

In 1970, Ornitol was tested on a larger scale. The cracked corn was pretreated by the Searle Company before shipment to us. Four areas were established: a control area, a test area using 0.1 percent Ornitol treated corn, and two 0.05 percent Ornitol treated corn areas. The corn was placed on platforms in the birds' breeding marshes from May 20 until June 17. Virtually all of the nests in these areas were checked regularly to determine hatching success. In the control area, in 30 nests observed, 62.9 percent of the eggs hatched; in the 0.1 percent test area, 46 nests were observed and 48.3 percent of the eggs hatched; in a 0.5 percent cattail nesting area where 11 nests were observed, 57.8 percent of the eggs hatched and in an 0.5 percent mallow nesting area where 25 nests were observed, 45.2 percent of the eggs hatched.

In 1971 the testing procedure was modified in an attempt to develop an approach that could be used by growers or wildlife control workers. The 0.05 percent Ornitol pretreated cracked corn was placed on the platforms for only two weeks in April, the period when the male Red-winged Blackbirds were establishing their territories. In the test area, 61 nests were observed with 47.7 percent of the eggs hatching. In the control area, 55 nests were observed and 52.6 percent of these

eggs hatched. This is normal hatching success, indicating that this treatment of birds with Ornitrol was ineffective. It appears that 0.1 percent Ornitrol pretreated cracked corn, administered for several weeks, offers the best hope for reducing bird numbers. No Ornitrol tests were conducted in 1972 or 1973.

Bird banding offers a way to study the movements of depredating species. It is useful to know if the birds that breed in an area are the same ones that cause the damage. In addition to banding nestling Red-winged Blackbirds, we erected four large walk-in decoy traps on Kent Island. About 1,000 birds were captured and banded in 1969. In the control area, 28 nestlings were banded. These were mostly Common Grackles. In 1970 only two traps could be operated. Only 138 birds (mostly grackles) were banded. In addition, 32 nestling Red-wings were banded. In 1971 the two traps were again operated, but only 15 birds were banded. In addition, 16 nestlings were banded. Banding was not continued in 1972 or 1973.

The third major activity undertaken was testing various techniques to frighten depredating birds from crops. We employed recorded Starling distress calls in 1969 to effectively exclude a flock of Starlings from an experimental vineyard in Baltimore County. A realistic plastic hawk suspended from a helium-filled balloon over corn fields proved difficult to handle and to keep elevated. After repeated attempts in 1969 and 1970 were unsuccessful, this technique was abandoned as impractical.

A much more effective technique has been the playing of an "Av-Alarm Chirper" in corn fields being damaged by blackbirds. This device generates disturbing sounds electronically. These sounds interfere with the birds' normal communication; they become confused and disperse. In 1969 and 1970, preliminary tests showed that this device did disperse the depredating birds from corn fields. In 1971, careful tests determined that of the many variations of sounds possible with this instrument, the most effective settings were at 3.8-4.2 khz with a down chirp at high volume. This was used in an on and off pattern; on for five seconds and off 10 seconds. In 1972, the system was expanded into four directional speakers, wired in parallel and mounted on folding tripods. The system was activated early in the morning and turned off at dusk. Results of the experiment were generally good. The Av-Alarm repelled the blackbirds from the fields while it was operating. It also effectively moved blackbirds from large roosts. In winter, 1973, a large roost in Montgomery County was dispersed by using this system.

Project No. H-87

OXIDATION OF ORGANOPHOSPHORUS INSECTICIDES IN MAMMALIAN SYSTEMS

Research on the mechanism and significance of biological oxidation of organophosphorus insecticides has continued. Efforts have been concentrated on elucidating the mechanism of dealkylation of the amide portion of certain organophosphorus compounds. The objective of this is to determine the relationship between these reactions and the toxicity of the molecules. An experimental compound, C-2307, which differs from previously investigated materials only in the substitution of a methoxy group for a methyl group on the amide, has been shown to be remarkably more toxic. After administration of C-2307 to rats and rabbits, it was ascertained that the basic route of metabolism of this compound was similar to that of dicotophos, a dimethylamide compound, but the rate was definitely lower. Information from metabolism studies has shown that the oxygen stabilizes the amide so that oxidative metabolism is inhibited. Most of the expected metabolites are isolated, however, so the increased toxicity of C-2307 must result from a lower rate of metabolism rather than the production of more toxic metabolites. It must be concluded, therefore, that oxidative dealkylation of these compounds is a detoxication mechanism.

Previously reported unknown metabolites of phosphamidon have been found to result from dechlorination and hydroxylation. These metabolites are the vinyl-hydroxy analog of phosphamidon, of desethyl phosphamidon and of the unsubstituted amide analog of phosphamidon. The mechanism of the replacement of the chlorine with a hydroxyl group remains a subject of active investigation.

In order to investigate the mechanism of metabolism of the substituted amide organophosphorus compounds, it was felt that *in vitro* systems would be useful. Cell cultures were investigated for their utility in metabolizing foreign compounds. Mouse fibroblast L-929 cells and Chang liver cells were not particularly useful for this purpose. However, primary human embryonic lung (HEL) cells were shown to metabolize a number of insecticide substrates very effectively. The level of oxidative activity paralleled the increase of proliferation of cellular protein. The oxidative metabolism of dimethoate, particularly, was very extensive.

The relationship between hepatic enzyme induction and metabolism and toxicity of organophosphorus insecticides has been studied. Injections of phenobarbital or feeding dieldrin to mice caused an increase in the activities of liver A-esterase, liver cytochrome P-450, and plasma B-esterase. Under *in vitro* conditions, phenobarbital and dieldrin also induced to oxidative and hydrolytic metabolism of dicotophos, dimethoate, and phosphamidon. The results suggest that increased dimethoate toxicity after treatments with inducers was due to stimulation of the activation of dimethoate to dimethoxon, while the increase in hydrolytic products after both pretreatments resulted in decreased toxicity of dicotophos and phosphamidon.

Project No. H-90

THE BIOLOGY AND CONTROL OF THE LESSER MEALWORM IN MARYLAND BROILER HOUSES

In 1966 virologists discovered the lesser mealworm could transmit the causative agent of acute avian leukosis to broilers. A survey of Maryland broiler houses showed a 39 percent infestation rate by the lesser mealworm. Because of the nearly complete lack of information about this beetle, a study of its biology was undertaken at the University of Maryland.

A semi-artificial medium was developed on which the beetles could be reared in the laboratory. A method of collecting eggs was developed. Eggs hatched in four days at 65 deg. F. Larvae transformed into adult beetles in about four months. Adult females laid eggs only after mating. Egg laying began about 13 days after mating. Each female laid an average of six eggs per day for one year. The average life span is about one year.

Lesser mealworms are a problem in broiler houses primarily where deep layering of litter is practiced. Such conditions foster a large population build-up, because of the longevity, fecundity, and defense secretions exhibited by the beetle. If the litter is removed after each broiler brood is sold, beetle populations will not build up to pest levels. Satisfactory chemical control was achieved with dimethoate, ronnel, and carbaryl.

Literature reports indicated the black fungus beetle, a species closely related to the lesser mealworm, also infested poultry houses. Therefore, taxonomic characters were found for separating late-stage larvae, pupae and adults of both species of darkling beetles.

Project No. H-92

POLLINATION OF CUCUMBERS BY HONEY BEES

For maximum yield and quality of cucumbers, effective pollination by insects is essential. Populations of native wild bees and domestic honey bees occur in most areas where cucumbers are grown. They have been considered by some to be adequate for the pollination needs of most cucumber crops. On Maryland's Eastern Shore, there was considerable doubt as to whether additional honey bee colonies should be moved into cucumber fields during the flowering period of the plant. Some growers felt that the additional cost of renting bees was not recoverable from increased yields.

During 1969 and 1970, experiments were conducted in the Salisbury, Maryland area to determine whether the placement of bee colonies in or adjacent to cucumbers resulted in any increase in yield or quality. The effect of adding no bees, one colony of bees for every four acres, one colony of bees per acre, and two colonies of bees per acre was evaluated. The results showed that although yield increases were not consistent as a result of additional honey bees placed in the field, the yields of producers using bees were in the order of two to three times the average yield for Maryland. More importantly, there was a strong correlation between the number of colonies added per acre and the frequency of mishap cucumbers that are downgraded, and subsequently of little value. During the two years of the study, the percentage of no. 2 (mishapen— cucumbers was 28, 18, 14 and 7 percent, respectfully, for the addition of 0, $\frac{1}{4}$, one and two colonies of honey bees per acre. On the basis of improved quality alone, the addition of two colonies of bees per acre is economically advisable.

Project No. H-93

STUDIES ON THE ALIMENTARY CANAL OF MOSQUITOES

Research on the alimentary canal of mosquitoes has shown that adult males and females visit certain fruits and flowers and probe and feed upon them.

Although males frequently land and walk on human skin, they never make any attempt to pierce the skin, even though they may lightly touch the skin with the tips of their proboscides. Female mosquitoes must be about one day old before they will make any attempt to feed on human beings. *Aedes aegypti* show a marked preference for man over a chick. Female mosquitoes never imbibe any sugary solution to the same extent as they imbibe blood. Female mosquitoes are capable of piercing the skin and taking blood when all but two of their legs have been removed. They are even capable of piercing the skin when all the legs have been removed: they do so while hovering. Females will take a second blood meal as soon as three hours after a first full meal.

Project No. H-94

INTEGRATED CONTROL OF ALFALFA WEEVIL

Beginning about 1966 and continuing to the present, the devastation of alfalfa by the alfalfa weevil has steadily declined. This decline was apparently brought about by the introduction of a number of natural enemies of the weevil that had not formerly occurred in the United States. At present, the alfalfa weevil is not considered as serious, on a statewide level, as it had been, although it is still capable of inflicting economic loss to growers of alfalfa throughout the state.

Since 1969, the research emphasis on alfalfa weevil has centered around ways of minimizing alfalfa weevil damage without injuring the new compliment of beneficial insects introduced. Essentially this has involved development of methods of early prediction of potentially serious populations so that only fields that would certainly be economically damaged would receive insecticidal treatments. The variety of insecticides available for effective control of these cases is increasing rapidly and offers growers many choices. His choice would depend on how much lead time he has before cutting his alfalfa. Some of the more desirable insecticides to use from the standpoint of effectiveness and minimal effect on beneficial insects must be used at an early date, hence early prediction of damaging populations is essential.

Results of the research thus far have enabled the development of a sampling procedure for use early in the year when alfalfa growth is in the three to six inch stage. From this sample it is possible to predict most of the time whether the field will need to be sprayed or not. In the few cases where this is not possible, a second or third sample may be necessary before the appropriate decisions can be made. The only problem with this procedure is that the sampling method requires expertise and technique not normally possessed by farmers and Extension agents. Current work is devoted to developing a simplified sampling method that can be used accurately by the farmer in order to enable him to make a decision to spray or not with confidence.

Project NO. H-95

HOST RELATIONSHIPS AND VIRULENCE OF SELECTED VIRUSES AFFECTING INSECTS

Determination of the Residual Life of a Nuclear Polyhedrosis Virus Applied for the Control of the Cabbage Looper

A nuclear polyhedrosis virus of the cabbage looper was applied to plots of broccoli and cabbage at the University Experimental Farm, Salisbury. Methods of sampling and extracting the virus from soil samples were devised so that residual activity could be detected when plots were sprayed at levels which have been found suitable for insect control. Soil samples were taken after each application and periodically until the plots were plowed before the next growing season. Each sample was then analyzed to determine the amount of infective virus remaining in the soil.

It was found that low levels of an infective nuclear polyhedrosis virus were present in all field plots before spraying was initiated, although no cole crops had been planted in the field for nine years. During the spray program, virus residues accumulated in the upper one-half inch of soil in all plots: It was noted that unsprayed plots contained as much infective virus as those plots sprayed with eight applications of 60 billion polyhedra per acre. Virus also continued to accumulate in both sprayed and unsprayed plots after completion of the spray schedule. This increase was attributed to the infection of cabbage loopers on the test plants. Virus residues persisted in the soil through the winter months. Large quantities of active virus were present in the spring before the field was plowed.

Further tests were performed to demonstrate that consumers eating raw cabbage obtain large amounts of these viruses annually. A periodic analysis of cabbage from unsprayed plots was conducted throughout the growing season. Laboratory bioassays with the cabbage looper, as the test insect, showed that significant quantities of virus accumulated on the leaf surface as a result of natural epidemics. The analysis of cabbage from five supermarkets near Washington, D. C. demonstrated that this epidemic generally begins in September, reaches a peak in October and declines during November. The concentration of viable virus on the head leaf surface of this cabbage during the peak of the natural epidemic was found to be about seven million polyhedra per square inch.

Laboratory studies were conducted to determine the effects of soil pH on virus longevity. Virus residues were found to be more rapidly inactivated at high and low pH with optimum persistence near neutrality. This indicates that heavy liming of farm lands may seriously shorten the effective life of applied virus.

Identification and Characterization of Nuclear Polyhedrosis Viruses of Insect Pests

The development of techniques to characterize and identify insect viruses was undertaken, since conventional methods used with other animal viruses were inadequate. It was determined that a soluble antigen from six nuclear polyhedrosis viruses would agglutinate the red blood cells of sheep, guinea pigs and adult chickens. The limits and optimum conditions for this reaction were defined to characterize the viruses. Attempts were made to differentiate closely related members of the group by the hemagglutination-inhibition test with antisera made against polyhedral inclusion body protein. This procedure was adequate in determining broad groups of viruses. It was found that sensitization of guinea pigs with nucleocapsid protein from each virus, followed by the use of an *in vitro* correlate of delayed hypersensitivity, was sufficient to differentiate the most closely associated viruses with no significant cross reaction. It is now feasible to complete a diagnosis within 24 hours of the receipt of a diseased specimen with the use of RNA extracts from the spleens of guinea pigs previously sensitized with specific viruses.

During the performance of the macrophage migration inhibition tests, it was found that a fraction of the polyhedral protein digest was capable of inducing a non-specific inhibition factor. It was determined that this factor acts as other known inducers such as the phytoagglutinins and concanavalin A, a synthetic compound. The function of this product relative to the induction of the antiviral substance, interferon, is being investigated, since a classical antigen-antibody mechanism has not been demonstrated in insects.

The Treatment of Insect Viruses for the Selection of More Virulent Control Agents

The low virulence of some insect viruses, which might have potential as microbial control agents, prompted an investigation to determine if virulence could be increased. Twenty compounds known to have mutagenic properties and two types of radiation were employed. Isolates of a nuclear polyhedrosis virus of the fall armyworm were treated and biological assays were performed to detect any significant changes in the activity of the virus. Mutants of increased virulence could not be selected from isolates treated with ultra-violet light, and microwave radiation sufficient to kill 90 percent of any test population did not produce results significantly different from those due to extraneous heating effects. Isolates treated separately, with two DNA base analogs and two coal tar derivatives resulted in a nine to sixteen fold increase in activity as judged by the decrease in dosage required to cause 50 percent mortality of the test insects. Also, a change in the rate of activity was indicated by a statistically significant decrease in the LT₅₀.

Project No. H-96

BIOLOGY AND CONTROL OF VEGETABLE INSECTS

Nine compounds were evaluated for control of imported cabbageworm and cabbage looper on Harris resistant Danish cabbage. Three of the compounds tested were biological control agents (two viruses and a *Bacillus thuringiensis*). The two viruses were quite effective for control of cabbage looper, but were weak in controlling imported cabbageworm. The *Bacillus thuringiensis*, or Dipel, was very effective in controlling both the cabbage looper and imported cabbageworm.

Also, Dipel was more effective than the other compounds in controlling the larger loopers. Although a weekly spray schedule is recommended for insect control on cabbage, it is often impossible to adhere to the schedule. Consequently, it is desirable to have a compound which will control the larger worms. Other compounds which gave superior control of cabbage looper and imported cabbageworm were Fundal, Lannate and Orthene.

In another experiment, two different sprayers were compared as to their effectiveness in controlling cabbage looper and imported cabbageworm. A high volume sprayer (85 gal/acre) and a low volume sprayer (10 gal/acre) were used. The low volume sprayer gave as good control as the high volume sprayer, when the row spacing was 36 inches. However, when the row spacing was reduced to 18 inches, the high volume sprayer gave superior control.

Two compounds were compared for their control of two-spotted spider mites on strawberries. Plictran gave control which was comparable to the more commonly used mixture of Kelthane and Tediion. It appears that Plictran offers longer control than the Kelthane plus Tediion.

Experiments designed to show the effectiveness of granular insecticides for control of Colorado potato beetles on direct seeded tomatoes resulted in several promising compounds. The following compounds gave good control of Colorado potato beetles: Temik, Furadan and Di-Syston.

Experiments on insect pests of sweet potatoes have shown that Furadan offers excellent control of gold bug. This compound also looks very promising in its control of flea beetle larvae.

Project No. H-97

BIOLOGY AND CONTROL OF SWEET CORN INSECTS

In the years from 1969 to the present, research work on sweet corn, primarily for processing, has been in several directions. Growers of processing corn are faced with economic disadvantages because of a low value crop. This means little can be spent for insect control. In order to increase production efficiency, there has been an extensive screening program to find better insecticides and biocontrol agents for use in insect control on sweet corn. As a result, two chemicals have been screened that are superior to anything in use today. One of these will probably be cleared for use in 1973.

There has also been work specifically directed to control the European corn borer. This insect attacks all parts of the corn plant, but processors and growers are mainly concerned with insects in the ears. Experiments have been underway to develop methods of predicting ear infestations of European corn borer and to develop techniques for directing sprays only at ear infesting borers.

A large scale program has been underway to evaluate commercial and experimental varieties of corn for resistance to insects. The characteristics that contribute to resistance to insects, and the varieties that have these characteristics, have been studied.

Corn is also damaged by insects that attack seed and small seedlings. Northern corn rootworm, armyworms and cutworms are some of the most serious of these. Research has been underway to screen available insecticides and determine their most effective method of use and application.

Project No. H-98

BIOLOGY AND CONTROL OF ORCHARD INSECTS AND MITES

During the past four years, nine new insecticides, three new fungicides, and eighteen new acaricides were evaluated in Maryland apple orchards for possible replacements for currently recommended pesticides.

The insecticides and fungicides were applied with low-volume spray equipment, either at 33X (10 gallons per acre) or at 10X (33 gallons per acre) and evaluated for insect and disease control, compatibility with other spray materials in the tank, and phytotoxicity to fruit or foliage. Two of these insecticides have been cleared for orchard use; prolate (Imidan) on apple, pear, peach, cherry and plum, and phosalone (Zolone) on apple and pear. Another insecticide, Phosvel, has an experimental label for grower trials on apple during the 1973 season. The remainder are still under test.

Two of the new fungicides, Topsin and Cela W-524 are still being tested, while the third, Elanco 273, has been shelved because of toxicological problems. Topsin and Cela W-524 show promise in the control of apple scab, *Venturia inaequalis*.

Acaricides were applied at 400 pounds pressure with a handgun. Four of the acaricides evaluated are now cleared for use in Maryland apple orchards. These are Galecron, Fundal, Plictran and Carzol. Evaluations will be increased on the effects these acaricides and others now in use have on orchard mite predators. Those found to be less detrimental to these mite predators will be recommended for use in the Maryland integrated mite control program.

In 1972, research was expanded to evaluate insecticides and fungicides for insect and disease control on French Hybrid wine grapes. The tests were under taken in a three acre vineyard near Hancock. Pesticides were applied at the rate of 100 gallons per acre with a Kinkelder mist sprayer. Azinphosmethyl (Guthion) and prolate were found to be effective in the control of most insects, except for Japanese beetle, *Popillia japonica* Newman. Carbaryl (Sevin) was necessary for the control of this beetle. Folpet (Phaltan) was found to be superior to benomyl (Benlate) in the control of black rot, *Guignardia bidwellii* (Ellis), Viala and Ravaz.

Project No. H-99

A NEW METHOD FOR APPLE SCAB CONTROL

A new concept in the control of apple scab, *Venturia inaequalis*, using Difolatan plus oil, or Difolatan alone in a single massive dosage application, was tested over a three year period. Difolatan has a strong retention quality and would redistribute to new growth with each rain. The single application was made in both dilute and low-volume spray equipment prior to bloom.

Data from these tests showed Difolatan plus oil, applied prior to green tip, or Difolatan alone applied prior to ¼-inch green, would control apple scab until petal fall. Difolatan plus oil applied after green tip caused severe foliage burn. Difolatan has no effect in the control of powdery mildew, *Podospharra leucotricha*, or cedar apple rust, *Gymnosporangium juniperi-virginianae*.

This technique was incorporated into the 1973 Maryland Spray Calendar for Commercial Apple and Peach Growers for apple grower trials. It may be of value to growers under the following conditions:

1. In those blocks which are usually wet in spring causing spray equipment to tear up the orchard floor.
2. In blocks which are a great distance from the main orchard or from a source of water.
3. When the grower has only one or two sprayers and cannot cover the entire orchard within the kick-back period of other fungicides.
4. In young non-bearing blocks to protect foliage until petal fall. Fungicides could then be added to insecticide sprays which are applied for control of leaf feeding insects.

This technique eliminated two to four pre-bloom sprays. However, it is essential for growers using this technique to start a seasonal fungicide program at petal fall. They should also check for insect or mite infestations prior to bloom and apply control measures if necessary. Difolatan may be used in low-volume applications, provided the spray equipment is properly calibrated and proper speeds are maintained to give wetting over the entire tree.

Project No. H-99

A NEW INSECTICIDE FOR PERIODICAL CICADA CONTROL

The new insecticide carbofuran (Furadan) manufactured by Niagara Chemical Company was found to be very effective in the control of Brood X of the periodical cicada, *Magicicada septendecim* L. This insecticide was applied in a dilute sprayer at 400 gallons per acre, and in a low volume sprayer at 10 gallons per acre. Control as a contact insecticide was about equal in both applications. However, the residual action carbofuran was greatest when applied as a dilute spray.

When carbofuran is cleared for use in commercial apple orchards, it will be used in place of carbaryl (Sevin) for the following reasons:

1. Carbofuran has a quicker knockdown and kill, both as a contact and as a residual spray, than does carbaryl.
2. The residual effectiveness of carbofuran is longer than carbaryl by several days.
3. No evidence has been reported that carbofuran acts as a fruit thinner. Carbaryl is a thinner for certain varieties of apples. The emergence of the cicada in 1970 occurred at the critical time for thinning apples with carbaryl. Carbaryl could not be applied until after June 1st on those varieties not needing a thinner, because of the danger of over-thinning. This was the day after egg deposition began. Some large growers had heavy twig damage because they could not completely cover their orchards until June 4th or 5th.

Project No. H-99

BIOLOGICAL DEGRADATION OF AGRICULTURAL INSECTICIDES

Earlier investigations of Mocap, a new organophosphorus insecticide-nematicide, in bean and corn plants have been extended to animals. Mocap-¹⁴C was administered to rats, after which the urine and feces were analyzed for metabolites. The rat urine contained methyl propyl sulfide, sulfoxide, and sulfone. The major water-soluble metabolite isolated from both plants and animals was O-ethyl S-propyl phosphorothioate, resulting from the removal of one propyl group from Mocap. Although ethyl propyl sulfide was an important metabolite in plants, it was not detected in rat urine, but rat urine was found to contain methyl propyl sulfide and its oxidation products. Methyl propyl sulfide may have been present in plants in extremely small amounts, but it was not an important metabolic product in plants. The striking difference between these two routes of metabolism in plants and animals may be partially explained by the fact that Mocap was completely metabolized in rats within six hours. On the other hand, unmetabolized Mocap was still present in significant quantities in beans up to 67 days after treatment, and in corn up to 100 days after treatment. The studies of Mocap metabolism in plants and rats have shown that the compound is rapidly converted in both systems to metabolites that are not likely to be considered toxic in any way.

Rats have been treated orally with a single dose of radiolabeled C-2307, an experimental organophosphorus insecticide, and with daily doses for 27 days. Rabbits were also treated daily for 16 days. The total recovery of radioactivity in the excreta of rats was comparable with what was found in earlier applications. In rat urine, 15 to 20 percent of the radioactivity was organoextractable, indicating potential toxicity, but extraction of rabbit urine yielded essentially no organoextractable radioactivity. Separation of the organoextractable radioactivity on silica gel thin layer plates resulted in six spots. Minute quantities of the parent compound were detected. In addition, monocrotophos and the unsubstituted amide analog were definitely characterized. The other three metabolites have not yet been identified.

Project No.-100

SURVEY AND EVALUATION OF MARYLAND FOREST INSECTS

This project was initiated in 1971 to develop a list of Maryland forest insects and to determine economic injury thresholds for some of the more important species. The checklist of Maryland forest insects is nearly complete and should be available by 1974. The work on economic injury levels has been concentrated on the Nantucket pine tip-moth, a serious pest of loblolly plantations. Results of the studies on pine tip-moth, although not yet complete, have yielded some valuable information. The tip moth attacks loblolly, Virginia and Scotch pine in Maryland, as well as some other less numerous species. It is far more damaging to young loblollies, since it causes a greater loss in tip growth. This is partly a function of the rapid growth rate of loblollies. There are two generations per year in central Maryland, and possibly three in southern Maryland. The first generation is the most damaging in general, although the second appears sometimes to be more numerous. With loblolly trees that are three to five feet tall in plantations, a population of a little over one moth per tree is sufficient to cause economic damage. A satisfactory method of estimating the populations has not yet been determined.

There are many natural insect enemies of the tip moth in Maryland, including parasites from 10 different families of Hymenoptera, plus several species of Tachinid flies and some Clerid beetles. Continued work on this insect should result in some definitive recommendations for managing loblolly and other susceptible pine plantations.

Project No. H-101

BIOLOGY AND CONTROL OF INSECTS AND MITES AFFECTING ORNAMENTAL PLANTS IN MARYLAND

According to state and federal horticultural specialists, scale insects are the most difficult insect pests of ornamental plants to detect, identify and control. Hollies and oaks are especially susceptible to scale insects. Studies are being made of the relevant pest species.

It has been determined that 12 species of wax scales occur in the U.S., including one new species. Illustrations, descriptions and a key for the identification of these species have been prepared. The common wax scale in Maryland is the ceriferus wax scale. The life history has been observed on holly and euonymus. This scale is parthenogenetic, has one generation a year in Maryland, and overwinters as an adult female. Oviposition begins in May, eggs hatch in mid-June and adults are present by late August. The insecticide *O,S*-dimethyl N-acetyl phosphoramidothioate was tested against all stages. It gave excellent control of crawlers, but poor control of the last three stages. Tests showed three insect juvenile hormone analogs give promise for excellent control of this pest.

The obscure scale attacks most species of oaks in Maryland, and pin oak appears most affected. We have discovered the life history of the obscure scale varies depending on whether the infested oak species is in the white oak or red oak group. While crawlers appear in July on pin oak, they do not appear until August on white oak. Therefore, sprays for crawler control must be applied in July or August depending on the oak species involved.

The time of crawler emergence can be predicted about two weeks in advance by observing the development of the exit flap by mature females. The obscure scale populations were somewhat held in check by eight species of parasitoid wasps, 14 species of predacious mites, and three species of lady-bird beetles.

Preliminary tests using three insect juvenile hormone analogs in sprays to control the euonymous scale on euonymus, the San Jose scale on pyracantha, and the wax scale on holly, indicate these chemicals may be as good or better than conventional insecticides now in use for control of these pests.

Project No. H-102

THE CIRCULATORY SYSTEM OF INSECTS

A series of studies have been made on the circulatory system of insects, with special reference to the functions of the heart of the American cockroach. The interest arises from the fact that the heart of this insect can be totally or partially removed without affecting its behavior or life span under ordinary laboratory conditions. It appears that the "reasons" this insect does not need a heart are (1) Insects do not use their blood to carry gases to and from the tissues (they have no red blood cells and no hemoglobin), (2) Most of their tissues are already well-supplied with nutriment so that they do not critically depend upon rapid or efficient circulation (in fact, rapid circulation in some stages of insects is not even desirable), and (3) In the absence of a heart, the blood is "stirred up" considerably by rhythmical contractions of the alimentary canal.

Project No. H-103

AQUATIC INSECTS IN MARYLAND

A review of recent literature dealing with behavior of mosquito larvae was published. Emphasis was placed on newer knowledge of feeding habits, orientation to the water surface, reactions to physical stimuli, formation of aggregations or clusters, and effects of overcrowding. Of particular interest are growth retardant factors produced by larvae of a given species which play a part in competitive displacement. In our laboratory studies of the effects of vibrations on *Aedes atropalpus*, larvae have been largely inconclusive. It has been found that *Aedes atropalpus* larvae can be forced to orient themselves to a bottom air-water interface. In one experiment 10 percent of the larvae pupated, however pupae did not adjust to the "up-side down" conditions. No adults emerged.

An investigation of mosquito vectors of *Dirofilaria immitis*, the causal agent of dog heartworm, has been initiated. This disease is known to be of increasing importance in Maryland. There is a "paucity of finite data on the actual transmission in nature". The nematode parasite is known to have a developmental cycle in mosquito vectors. At a kennel in Prince George's County where the infection rate was determined to be 51 percent in 1971, mosquitoes have been trapped and held in the laboratory to allow parasites to complete their development. Dissections have revealed the presence of small larvae in two mosquito species, second-stage larvae in one species and third stage or infective larvae in two species. Attempts are being made to show that *Culex salinarius* is capable of actual dog-to-dog transmission. If a particular species of mosquito can be incriminated, the disease could be controlled by reducing the population level of the known vector. The critical phase of the work involves holding infected mosquitoes and inducing them to take a second blood meal.

In addition to field work, the problem is under attack in the laboratory. Dogs are being maintained under controlled conditions, and colonies of *Culex salinarius* are also maintained. Results, so far, clearly indicate that this species of mosquito feeds on dogs.

Over 400 microscope slides of Maryland mosquito larvae were prepared in 1972. These are useful for reference purposes. Work is continuing on a reference collection of adult mosquitoes.

Four wetland areas have been selected as stations for intensive and regular insect collecting leading to an inventory of insects associated with the Chesapeake Bay. The marshes are located at Deal Island, Kent Island, Magruder's Landing on the Patuxent and Gunpowder State Park. The principal objective of this phase of the project is to acquire an understanding of the normal or average insect fauna in and adjacent to selected marshes. Comparisons of insect populations in different ecological areas and at different seasons are being made. Relationships of insects to other forms of life and the economic importance of insects in wetlands are being investigated. Marshes are visited routinely on a bi-weekly schedule during the warmer months of the year. Most collections are made by netting and dipping, but tabanid (deer-fly) traps have been operated. Thousands of specimens have been collected and processed for study. Identification is time-consuming, but progress is being made. It is estimated that approximately 50 insect families are represented in material collected. It is not yet possible to estimate the number of species. In the order Diptera (true flies), over 130 species in over 30 families have already been identified. Many of these constitute new records for Maryland, and one species is probably undescribed -- that is, new to science. Most of the material in the orders Odonata, Orthoptera, Neuroptera and Diptera has been identified to the species level. During the summer of 1973 emphasis will be placed on collecting and evaluating the economic importance of deer flies and other tabanids which, except for mosquitoes, are the most noxious insect pests in the wetland areas. As a result of surveys and identifications, it is expected that baselines will be established and that these data will serve a very useful purpose. Currently base-line information is very incomplete. As changes in the marsh environment occur, the effect on the insect fauna cannot be accurately measured. It is desirable to have an inventory for purpose of comparison and contrast.

Project No. H-104

ENHANCEMENT OF DISEASE RESISTANCE IN SOYBEANS AND SUPPRESSION OF MYCOTOXINS IN CONTAMINATED SEED

Enhancement of Disease Resistance

Phytoalexins, the antifungal antibiotics produced by plants in response to challenge by certain parasites, may provide a high degree of resistance when produced at the infection site in concentrations large enough to inhibit the parasite. However, the ability of the challenger to stimulate phytoalexin production, the ability of the host plant to produce phytoalexin, and the degree of sensitivity of the challenger to the phytoalexin are all genetically controlled. In many host-parasite interactions, the phenotypic expression of these three genetic systems is such that resistance does not occur. Various physical and chemical means of inducing phytoalexins have been developed. Some of these techniques permit the stimulation of concentrations of phytoalexins high enough to bring about resistant reactions in host-parasite interactions in which the phenotype would normally have been expressed as susceptibility. Hydroxyphaseollin (HP), a phytoalexin from soybean (*Glycine max*), has been induced by treatment of plant tissues with ultraviolet irradiation, with various chemicals, and by inoculation with fungi and viruses.

Young soybean plants exposed for 30 minutes to ultraviolet irradiation ($\lambda_{\text{max}} = 253 \text{ nm}$) and maintained in darkness contain high concentrations of HP after 24 hours, and maximal concentrations after 96 hours. When genetically susceptible plants are irradiated, they become much less susceptible to the fungal soybean pathogen, *Phytophthora megasperma* var. *sojae*. Ultraviolet induction of HP is photoreversible. Irradiated plants must remain in darkness for at least 48 hours for maximal production of HP. Treatment with amounts of ultraviolet irradiation sufficient to induce protective concentrations of HP also cause extensive damage to the plants.

HP can also be induced by incubation of soybean tissues in a wide variety of chemicals; some of them in concentrations low enough to cause little or no visible phytotoxicity. Amines, or compounds that release amines upon decomposition, are among the most effective chemical inducers of HP. Soybean cotyledons or hypocotyls incubated for 12 hours in a 10^{-3}M solution of butylamine produce enough HP to completely inhibit germination of conidia of *Cladosporium cucumerinum*. Similar concentrations of butylamine do not inhibit germination of conidia, nor are they visibly phytotoxic to soybeans. Several fungicides contain amines or produce decomposition products containing amines. Many of these fungicides are effective inducers of HP and part of their effectiveness under field conditions may possibly be credited to their ability to induce phytoalexins. When butylamine is applied to roots of genetically susceptible soybean plants, the hypocotyls become less susceptible to the soybean pathogen, *P. megasperma* var. *sojae*. Inoculated hypocotyls of butylamine-treated plants of either resistant or susceptible varieties also contain considerably more HP than inoculated plants of the same variety not treated with butylamine. Neither butylamine nor HP can be detected in hypocotyls of wounded or non-wounded uninoculated plants, however, suggesting that butylamine applied to roots does not induce HP in the hypocotyls but conditions hypocotyls to produce more HP when challenged by a fungal invader.

Occurrence of Aflatoxins in Soybeans

Above average rainfall in Maryland during August, September and October of 1971 resulted in heavy mold growth in soybeans still in the field. Although aflatoxins are not considered a serious problem on soybeans, of 28 samples of soybean seed tested, aflatoxin were found in 14 – two of which had been used in poultry feed. There was no correlation between the percentage of moldy grain and the concentration of aflatoxins. Even though aflatoxin-producing species of *Aspergillus* spp. could be isolated from soybean seed, detectable amounts of aflatoxins were not always present. This study indicates that soybeans can be a suitable substrate for growth of *Aspergillus* spp. and resultant aflatoxin production, especially when heavy rains occur during seed maturation.

Control of Aflatoxin Production by *Aspergillus* spp.

Dimethyl sulfoxide (DMSO) can control aflatoxin production by *Aspergillus* spp. both in liquid culture and plant tissue. In liquid culture, similar amounts of aflatoxins B₁, B₂, G₁ and G₂ were produced in liquid cultures containing no DMSO (control), 5,000 ppm or 10,000 ppm DMSO. Only aflatoxins B₁ and G₁ could be detected in filtrates of cultures grown in medium containing 25,000 ppm DMSO; no aflatoxins were detected in filtrates from cultures of *A. flavus* grown in 45,000 ppm and 50,000 ppm DMSO. When peanut seeds, used as a substrate for *A. flavus*, were treated with different concentrations of DMSO prior to inoculation, both pigmentation of the fungus and aflatoxin production by the fungus were affected. As the level of DMSO was increased, pigmentation of the conidia changed from normal green to a pale green and eventually white. Although a concentration of 0.62 percent DMSO caused a slight increase in total aflatoxin level; treatment with higher DMSO concentrations decreased the level of aflatoxin. Extracts from soybeans treated with 2.5 percent DMSO contained approximately 62-64 percent less aflatoxin than those treated with water. Possibly DMSO could be used alone or with other compounds to prevent formation of aflatoxins in plant tissue.

Aspergillus niger, which does not produce aflatoxins, also detoxified aflatoxins present in culture filtrates. The use of *A. niger* may offer an alternative means of preventing aflatoxin production other than by control of the aflatoxin producing fungi by use of pesticides.

Project No. J-104

MOSQUITO BIOLOGY

Aedes atropalpus is a mosquito which spends its immature stages in rock holes, such as those found at the Great Falls of the Potomac. Eggs are deposited above the water level in rock holes. When the water level rises after rains, eggs will usually hatch if the water is warm enough, but the eggs of this species are very resistant to unfavorable environmental conditions. Eggs remain viable for many months. Under laboratory conditions, studies were conducted to determine the length of time eggs remain viable when subjected to selected, carefully measured temperatures and humidities. It was found that temperature is a much more important factor than relative humidity in influencing the survival of *A. atropalpus* eggs.

In cooperation with the Walter Reed Army Institute of Research, studies were conducted on the hybridization of three laboratory strains of *Anopheles stephensi* Liston. Differences in fertility were attributed mainly to variations in decreased or increased egg development or hatch rate or both. The findings have been discussed with respect to taxonomy, population genetics, evolution and possibilities of genetic control of the species.

Studies were concluded on the feeding behavior of six common Anopheline mosquitoes of West Pakistan. Consideration was given to host preferences, population dynamics, nocturnal feeding behavior and endophilic tendencies. Concurrent biting and concurrent bait traps demonstrated the willingness of *Anopheles nigerrimus* to feed on man in the presence of bovine hosts. Precipitin tests yielded a zero anthropophilic index. This species was found to be exophilic. The work was done in cooperation with the Institute of International Medicine of the University of Maryland School of Medicine and the Maryland State Board of Agriculture.

Records of mosquito collections made during a 12-year period were assembled. Distributional patterns and relative abundance of species in three physiographic areas of Maryland were analyzed. Of the 53 species known to occur in Maryland, 12 are considered to be common, and 23 are considered rare. Several other species are locally abundant at certain times. There are 17 species known to occur only in the Coastal Plain area, below the Fall Line. Only two species have been collected exclusively above the Fall Line. These studies resulted in the publication of new state records for five species. Outside of localities where State Board of Agriculture Mosquito Control operations occur, a relatively insignificant amount of collecting has been done. There are several unique ecological areas in the state such as the Finzel Swamp in which unusual species of mosquitoes are found.

Project No. H-73a

BIOLOGY AND CONTROL OF TOBACCO INSECTS

The main effort with tobacco insects has been an ecological study of the tobacco flea beetle. Observations on its seasonal activity indicate two seasonal peaks in its occurrence, one shortly after planting and one in mid-August prior to cutting. The mid-August peak is the larger. Flea beetles prefer the leaves on the lower half of the plant. Experiments with varieties to determine flea beetle preference indicate that the three major Maryland varieties of tobacco, Catterton, Maryland 10 and Maryland 609 are attacked equally by flea beetle.

Experiments are currently being conducted to observe populations of larvae in the soil. Attempts are being made to determine the extent the larvae feed on tobacco roots, to correlate the populations of larvae in the soil with the beetles feeding on foliage, to determine the effects of cultural practices on populations of flea beetles, and to determine overwintering sites and overwintering survival rates of flea beetles.

Project No. H-74

COMPARATIVE MORPHOLOGY AND PHYSIOLOGY OF INSECT BLOOD CELLS

Studies were made on the comparative morphology and physiology of insect blood cells in an attempt to match their structure with their function. This was a long-range project that was terminated in 1971 for lack of funds. Insects totally lack red blood cells and possess only white blood cells (hemocytes). Through these studies, entomologists now have a single uniform classification of cells and detailed information relative to changes in types and numbers with the major events in the life of representative insects. It was revealed that there are four major functional types of cells: stem cells, phagocytic cells, coagulocytes and cells which secrete specialized enzymes. Stem cells can develop into all types and are the progenitors for them. Phagocytic cells also engage in nutritional activities. Coagulocytes function only during wounding.

Project No. H-76

INSECTICIDE RESIDUES IN SOIL AND RAW AGRICULTURAL COMMODITIES

In order to facilitate the replacement of the chlorinated hydrocarbon insecticides with less persistent materials, research on the behavior of organophosphorus insecticides in soils has been underway. The analysis of soil samples from two different types of soil treated with granular disulfoton, an organophosphorus insecticide, has shown that disulfoton is somewhat more persistent in sandy loam than in loamy sand. When spinach was grown in loamy sand, no residues of disulfoton could be detected at harvest 64 days later. Residues were detected in small quantities in spinach grown in sandy loam at 56 and 63 days after treatment, but not later.

The effect of soil type on the rate of disappearance of dimethoate has also been studied. Three soil types were compared using two crop plants, beans and corn, at two different times of the year. The soil types were silty loam, clay loam and loamy sand, ranging in pH from 5.8 to 6.4, and in organic matter from 0.5 percent to 2.1 percent. Dimethoate disappearance did not seem to be influenced by pH, but was somewhat slower in the higher organic matter soils. The rates of dissipation of dimethoate and its active metabolites from the three soil types were less than three days in each case. Dimethoate was more persistent under dry soil conditions than in wet soil. The differences in rate of dissipation and movement between the three soil types were minor, and were not statistically significant.

BHC was applied to winter spinach on the Eastern Shore of Maryland for control of the green peach aphid, a difficult to control pest. Residue analyses were made using electron capture gas chromatography. It was noted that the results were quite erratic between the various treatments, indicating the possibility of drift, spray overlap, and considerable volatilization of BHC. The residues were below 1.0 ppm by seven days after treatment, and below 0.2 ppm by 14 days after treatment. However, detectable residues were found as long as 133 days after treatment (maximum 0.035 ppm).

Analyses of commercially frozen and canned vegetable and fruit samples have been continued to ascertain that Maryland insecticide recommendations are not resulting in illegal residues in vegetables. No samples have been found in which the insecticide residue levels exceeded tolerances.

Project No. H-67

PHYSIOLOGY OF INSECT REPRODUCTION

A long series of investigations have been made dealing with the general physiology of reproduction in insects, with special reference to mosquitoes. The importance of such studies lies in the fact that the ideal way to control an insect would be to interfere with its ability to reproduce (e.g., by releasing sterile males). In order to understand why such methods will or will not work in practical field tests, it is essential to have detailed laboratory information. We have been studying the sexual activities of free-flying mosquitoes in various size cages and have discovered many new features which may explain why the sterile male release of mosquitoes will not work in nature. For a long time it was believed that *Aedes aegypti* mosquitoes mated only once and that once the female had been inseminated she would quickly become "unattractive". This phenomenon is true with forced and tethered matings. It has been discovered in this laboratory, however, that free-flying females are mated with many times, and that they are *not* rendered "unattractive" by insemination. Further, it has been found that males after inseminating about five females will continue to copulate with other females, but will no longer inseminate them, even though they have replenished their sperm supply. Individual males do not court or copulate well when isolated: they perform much better in small groups. Further, it has been found that some males are better copulators than others: that is, they are highly competitive in mating. The most highly competitive of the males continues to out-copulate other males long after he has been the first to run out of sperm. The males, when continuously introduced to females, eventually become non-coital: they continue to chase females, but do not copulate with them.

Project No. H-72

DO NOT CIRCULATE

In addition to state and federal funds, the research program of the University of Maryland Agricultural Experiment Station has received support during the year from many public, private and industrial organizations and individuals. It is regretted that space does not permit recognition of all sources of help, but the cooperation of all is herewith gratefully acknowledged.

